EXHIBIT B

PI: **VOSKUHL, RHONDA R**	Title: Neurodegeneration Underlying Distinct Disabilities in Multiple Sclerosis Using a Cell-Specific, Region-Specific, and Sex-Specific Approach	
Received: 07/08/2022	FOA: NS22-038	Council: 01/2023
Competition ID: FORMS-G	FOA Title: Research Program Award (R35 Clinical Trial Optional)	
1 R35 NS132150-01	Dual:	Accession Number: 4732963
IPF: 577505	Organization: UNIVERSITY OF CALIFORNIA LOS ANGELES	
Former Number:	Department: Neurology	
IRG/SRG: ZNS1 SRB-H (26)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 591,985 Year 2: 591,985 Year 3: 591,985 Year 4: 591,985 Year 5: 591,985 Year 6: 591,985 Year 7: 591,985 Year 8: 591,985	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Rhonda Voskuhl MD	The Regents of the University of California, Los Angeles	PD/PI
Yuichiro Itoh PhD	The Regents of the University of California, Los Angeles	Co-Investigator
Jin Zhou PhD	The Regents of the University of California, Los Angeles	Co-Investigator
Allan MacKenzie-Graham PhD	The Regents of the University of California, Los Angeles	Co-Investigator
Prabha Siddarth PhD	The Regents of the University of California, Los Angeles	Co-Investigator

Additions for Review

Accepted Publication	Accepted Publication
Other	Accepted Publication and Late Breaking Data

OMB Number: 4040-0001
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED 2022-07-08

Application Identifier

5. APPLICANT INFORMATION **UEI*:** RN64EPNH8JC6

Legal Name*: The Regents of the University of California, Los Angeles
Department:
Division:
Street1*: Office of Contract and Grant Administration
Street2: 10889 Wilshire Boulevard, Suite 700
City*: Los Angeles
County: Los Angeles County
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90095-1406

Person to be contacted on matters involving this application
Prefix: First Name*: Lydia Middle Name: Last Name*: McHam Suffix:
Position/Title: Contract and Grant Analyst
Street1*: 10889 Wilshire Boulevard, Suite 700
Street2:
City*: Los Angeles
County: Los Angeles County
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90095-1406
Phone Number*: (310) 794-0167 Fax Number: Email: lydia.mcham@research.ucla.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-956006143-A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Neurodegeneration Underlying Distinct Disabilities in Multiple Sclerosis Using a Cell-Specific, Region-Specific, and Sex-Specific Approach

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2023 03/31/2031

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-033

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Rhonda Middle Name: R. Last Name*: Voskuhl Suffix: MD
Position/Title: Professor
Organization Name*: The Regents of the University of California, Los Angeles
Department: Neurology
Division: David Geffen School of Medicine
Street1*: 635 Charles E. Young Drive South
Street2: 475D Neuroscience Research Bldg 1
City*: Los Angeles
County: Los Angeles County
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90095-7334
Phone Number*: (310) 206-4636 Fax Number: (310) 206-9801 Email*: rvoskuhl@ucla.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$7,307,976.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$7,307,976.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Ms. First Name*: Jessica Middle Name: Last Name*: Kim Suffix:
Position/Title*: Senior Contract and Grant Analyst
Organization Name*: The Regents of the University of California, Los Angeles
Department: Office of Contract & Grant Adm
Division:
Street1*: 10889 Wilshire Boulevard, Suite 700
Street2:
City*: Los Angeles
County: Los Angeles County
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90095-1406
Phone Number*: 310-983-3673 Fax Number: Email*: jessica.kim@research.ucla.edu

Signature of Authorized Representative*
Jessica Kim

Date Signed*
07/08/2022

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter1071187008.pdf

424 R&R and PHS-398 Specific
Table Of Contents

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The Regents of the University of California, Los Angeles
UEI:	RN64EPNH8JC6
Street1*:	635 Charles E. Young Drive
Street2:	GNRB 479, 476, 480, 484; lab bays 21-24
City*:	Los Angeles
County:	Los Angeles
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90095-7334
Project/Performance Site Congressional District*:	CA-033

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The Regents of the University of California, Los Angeles
UEI:	RN64EPNH8JC6
Street1*:	1100 Glendon
Street2:	Suite 1820
City*:	Los Angeles
County:	Los Angeles
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90095-7394
Project/Performance Site Congressional District*:	CA-030

Project/Performance Site Location 2

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Regents of the University of California, Los Angeles
UEI:	RN64EPNH8JC6
Street1*:	635 Charles E. Young Drive
Street2:	GNRB 225Y, 225Z, 225Z1
City*:	Los Angeles
County:	Los Angeles
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90095-1769
Project/Performance Site Congressional District*:	CA-033

Additional Location(s) File Name:

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number A3196-01

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ● **Yes** ❍ **No**

6.a. If yes, identify countries: Germany

6.b. Optional Explanation: See attached other attachment on foreign justifications

	Filename
7. Project Summary/Abstract*	R35_Abstract1071243889.pdf
8. Project Narrative*	Project_Narrative1071122653.pdf
9. Bibliography & References Cited	Reference_Cited1071243920.pdf
10.Facilities & Other Resources	Facilities_Resources1071122655.pdf
11.Equipment	Equipment1071122656.pdf
12. Other Attachments	Bibliography_Voskuhl1071243921.pdf R35_Mentoring1071243922.pdf Foreign Justification.pdf

Multiple sclerosis (MS) is an autoimmune, neurodegenerative disease with inflammation, demyelination, axonal damage, glial activation and synaptic loss. There are relapses and permanent disabilities. Despite success of treatments targeting cells in the immune system, there is an unmet need for treatments targeting cells in central nervous system (CNS) to repair disabilities. Four observations provide rationale for a new approach to neurodegeneration in MS: 1) MS patients are heterogenous in their disabilities, and distinct disabilities (walking, vision, cognition, coordination) are served by different CNS regions, 2) Even in healthy brain, a given CNS cell type differs in gene expression from one brain region to another, 3) Being female versus male impacts disability worsening, and 4) Aging aligns with disability progression. Here, we will use a cell-specific, region-specific, and sex-specific approach to discover optimal treatment targets for distinct disabilities in MS women and men.

Bedside to Bench to Bedside in MS: Clinical observations of sex differences are investigated at the preclinical level then translated back to the clinic as trials designed for each sex. Preclinical use of female and male mice with experimental autoimmune encephalomyelitis (EAE) entails *in vivo* MRI for region-specific atrophy, neuropathology of each region, RNA-sequencing of distinct CNS cells in each region, immunohistochemistry validation of top genes in highly differentially expressed pathways, cell-specific conditional knockout (CKO) of target genes to reverse phenotype, and knockdown of target genes with pharmacologic treatment to reverse phenotype. The effect of genetic (CKO vs WT) and/or pharmacologic (treatment vs placebo) interventions on reversal of gene expression is determined in each sex. Human MS data guide preclinical research at three checkpoints: i) MRI in females and males with MS revealing sex differences in substructure atrophy prioritize regions in EAE with atrophy, ii) Single nuclei RNA-seq analyses in females and males with MS revealing gene pathways of interest prioritize gene pathways in EAE, iii) immunohistochemistry in females and males using MS postmortem tissues validate immunohistochemistry in EAE. Substitution of use of female versus male mice (as above) with use of gonadectomized versus gonadally intact mice will reveal activational effects of sex hormones. Use of Four Core Genotype mice will reveal sex chromosome effects versus developmental hormone effects. Use of young versus old mice will reveal the effect of aging.

This R35 proposal will: 1) Extend our **cell-specific and region-specific transcriptomics** in astrocytes and oligodendrocytes to microglia and neurons, with cell:cell interactions revealed in mice double-labelled to show gene expression changes in two distinct cell types in the same region in the same mouse, and 2) Determine if there are effects of **sex** and/or **age** on the most differentially expressed cell-specific and region-specific pathways. In summary, this R35 proposal takes our research to the next level: Identifying sex by age interactions in cell-specific and region-specific transcriptomics, neuropathology, and substructure atrophy on MRI.

The greatest unmet need in multiple sclerosis (MS) is to develop novel treatments targeting cells and processes within the central nervous system (CNS) to confer neuroprotection and repair disabilities, in not only relapsing remitting MS, but also in secondary progressive MS. A “one size fits all” neuroprotective treatment approach in MS will not work, since 1) MS patients are heterogenous regarding which disabilities are predominant, 2) being female versus male impacts rates of disability progression, and 3) aging corresponds with disability progression. This R35 will use a cell-specific, region-specific, and sex-specific approach to discover neurodegenerative targets optimized for each disability in MS models in females and males during young adulthood and aging.

FACILITIES AND OTHER RESOURCES

PROJECT SCIENTIFIC ENVIRONMENT

University of California, Los Angeles*. Home Institution of all investigators.*
UCLA has outstanding the expertise in all the areas required in this proposal, namely neuroimmunology, neuroscience, bioinformatics, neurogenomics, and neuroimaging. Together this creates an environment of highly complementary areas of expertise, thereby assuring success of this challenging project.

FACILITIES & RESOURCES

Laboratories / Office / Computing

Dr. Rhonda Voskuhl: Dr. Voskuhl's private office is in the Neuroscience Research Building, 4th floor, in the center of the UCLA Medical Campus. It is a southwest corner office 479C (30x20 feet) and houses 2 Mac computers. The Voskuhl labs and consists of four rooms (479, size 28x40 ft; 476, size 22x30 ft; 480, size 22x30 ft. and 484, size 28x30 ft) and dedicated mouse rooms with, while her dedicated mouse rooms include Rooms 420A and 420K. 479 is lab bench space for histology, FACS staining, PCR, preparation of total RNAs from tissues and cells, polyA+ RNA isolations, gel electrophoresis, etc. It contains 10 desktop computers and also has a chemical hood. Room 476 is used for murine cell culture and incubation and contains 2 sterile hoods. Room 480 is used for human PBMC preparation, generation of TCLs, and incubation, proliferative assays and ELISAs. It has one sterile hood and a Beta plate counter with cell harvester. Room 484 is dedicated to immunofluorescence and electrophysiological studies. Room 420A is Dr. Voskuhl's mouse procedure room. It is used for immunizations, behavioral testing, the dissection of mice, adoptive transfers, eye bleeds, etc. Room 420K is Dr. Voskuhl's mouse return room where mice with EAE are returned for housing to permit frequent behavioral testing. Mice are bred in a barrier facility on the basement floor of RB1. All mice being generated or received from a vender prior to experiments are housed in this common barrier facility. ***Animal Housing and Care.*** Animal care is provided by the Division of Laboratory Animal Medicine at UCLA, which is an approved AAALAC facility. In total Dr. Voskuhl's animal space can house approximately 1300 mice at any given time. Her lab has a special mouse procedure room (#420A) that is available for surgeries (immunizations, dissections), behavioral analyses, and adoptive transfers. Dr. Voskuhl's lab also includes a mouse return room (#420K) where mice are returned for housing following procedures. A common barrier facility on the basement floor of the same building for breeding, housing and routine care and disease surveillance.

Dr. Yuichiro Itoh: Dr. Itoh's private office is in the Neuroscience Research Building. Dr. Itoh's office is in 479B (20x20 feet). It houses 2 computers for bioinformatics analyses of transcriptomes. Dr. Itoh uses the Voskuhl lab for wet lab work to complement his computational analyses identifying novel targets. His office is adjacent to Dr. Voskuhl's office.

Dr. Jin Zhou: Dr. Zhou will provide gene expression and methylation statistical analyses, as well as providing access to the database for dissemination to the public. As an Associate Professor in the Department of Medicine Statistics (DOMStat) Core, UCLA Division of General Internal Medicine and Health Services Research, she has full access to all of the resources and staff in the DOMStat Core. Her office is in the DOMStat floor of 1100 Glendon Avenue, Suite 11820, a block away from the UCLA main campus.

Dr. Allan MacKenzie-Graham: Dr. MacKenzie-Graham's private office is adjacent to his laboratory. The MacKenzie-Graham laboratory comprises three rooms in the Neuroscience Research Building Facility 2nd floor (see below). The wet lab (225Z) is approximately 225 square feet with bench space for perfusion, sample preparation, optical clearing (CLARITY), histology, immunohistochemistry, gel electrophoresis, and hybridization of blots. Dr. MacKenzie-Graham's office space is 96 square feet (225Z-1), adjacent to the lab. A postdoctoral office space of 76 square feet (225Z-2) is also adjacent to the lab. Individual compute resources are described in the Equipment section. As a member of the Ahmanson-Lovelace Brain Mapping Center faculty, Dr. MacKenzie-Graham has unlimited access to the compute and storage resources described below.

Dr. Prabha Siddarth: Dr. Siddarth will provide high-level statistical analyses to the investigators and she has access to SIStat, the biostatistics core in UCLA's Semel Institute for Neuroscience and Human Behavior. SIStat provides affiliated projects with software tools and direct services such as database creation and management and quality assurance. Dr. Siddarth's office space is 125 square feet (Suite 37-444), and adjacent to her office is a large office space to house graduate students, postdoctoral fellows and interns. As a

member of the Semel Institute's research faculty, Dr. Siddarth has unlimited access to many high performance computer systems in the Semel Institute.

UCLA Core facilities, all of which are readily available for this proposal:

UCLA Informatics Center for Neurogenetics and Neurogenomics (ICNN)

UCLA Department of Medicine Statistics Core (DOMStat)

UCLA Semel Institute Biostatistics Core (SIStat)

Mitochondrial function / Synaptosome Core

UCLA Brain Mapping Core for Neuroimaging

The Neuroscience Research Building Facility houses the research MRI scanners (PRISMA for humans and the 7T for mice):

The faculty, staff and major physical resources to be used by this project are housed in the Neuroscience Research Building Facility. The NRB Facility resides within the Department of Neurology at the UCLA School of Medicine. The facility was designed and furnished for the acquisition, processing, and storage of brain image data from a variety of sources.

Physical Infrastructure: The facility is physically located in the Neuroscience Research Building (NRB), occupying approximately 8,425-ft² of the building's second floor. The space is comprised of a reception area, Director and faculty offices, a modern computer room, a user area, wet labs, a conference room, and the Data Immersive Visualization Environment (DIVE) discussed here within. The data center in this space includes a raised floor, a 130 KVa UPS/PDU capable of providing uninterruptible power to all equipment housed in the room, dual Leibert air conditioning units, humidity control, and a fire suppression mechanism. A sophisticated event notification system is integrated in this space to automatically notify personnel of any abnormal power, water, or HVAC issues that arise. Immediately adjacent to this machine room is a user space with eighteen individual stations separated by office partitions. Each station is equipped with a four copper Gigabit network ports and 802.11n wireless signals for networked image processing, visualization and statistical analysis. To provide telephony services, the facility relies on Voice over IP (VoIP) technology. VoIP units, including wireless handsets, are distributed throughout the laboratory. In addition, the conference room and the DIVE are equipped with supplementary H.232 videoconferencing systems.

Having reached the power, cooling, and physical capacity limits of the second-floor data center, the facility was augmented with an additional 2,127-ft² space on the building's first floor containing a state-of-the-art data center and additional offices. The office space can support up to twenty researchers and is outfitted with the same technological specifications as the existing facility. The 580-ft² data center is outfitted with an "in-row" cooling solution that regulates temperatures strictly within the server racks—as opposed to the traditional raised floor, hot-cold aisle technique that uses more energy to cool the entire room. Multiple chilled water lines are fed from the building to a Rittal TS 8 rack system, which houses the servers and provides in-row cooling. The racks are fully enclosed and deliver chilled air to the front of the machines while recycling warm air exhausted at the rear. During normal operation, keeping the air within the system provides maximum efficiency, but should a component fail, once temperatures reach 80°F the magnetic locks on the rack doors are programmed to release, forcing the doors open to provide ambient air to the machines. A 550KVa Eaton UPS provides uninterruptable power to the facility's current equipment and is sized to accommodate future large hardware expansions. In order to support the additional personnel and computational resources, the data center is equipped with multiple 10 Gigabit connections in a fault-tolerant, load-balanced configuration that interconnect with the primary data center.

Finally, a tertiary 800-ft² data center, located in UCLA's Reed Building, is shared with the Department of Neurology. This physically separated building primarily houses tape-based backup systems, to provide a failsafe data backup in case of a catastrophic event at NRB. This third data center is connected to our primary by dual, redundant Gigabit fiber connections as well as dual single-mode fibrechannel connections to extend the facility's storage area network (SAN).

Compute Resources: Rapid advancements in imaging technology have provided researchers with the ability to produce very high-resolution, time-varying, multidimensional data sets of the human brain. The complexity of the new data, however, requires immense computing and storage capabilities. To meet the computational requirement, the NRB Facility houses the following high-performance computing (HPC) clusters:

- a 306-node Oracle V20z cluster. Each compute node has dual 2.4GHz AMD Opteron processors with 8GB of memory (612 cores total)
- an 80-node Oracle X2200 M2 cluster. Each compute node has dual 2.2GHz quad-core AMD Opteron processors and 16GB of memory (640 cores total)
- a 416 node HP ProLiant SL2x170z G6 cluster. Each compute node contains dual quad-core 2.66GHz Xeon 5500 processors and 24 gigabytes of memory (3,328 cores total)

To augment the facility's cluster resources, a group of 8 HP DL580 G7 32-core 2.4GHz X7550 machines, each with 256GB RAM, provide the high-memory computing requirements needed for biomedical applications, particularly gene sequencing.

Storage Resources. The NRB facility is architected using a fault-tolerant, high-availability systems design to ensure 24/7 functionality. To complement its computational systems, the laboratory uses high performance network attached storage (NAS) and SAN technologies to accommodate current and projected storage requirements. The facility’s current capacity is approximately 4 petabytes of online and offline storage.

To meet the extensive I/O demands of the laboratory’s HPC clusters and address the bottlenecks inherent in traditional NAS technology, the facility has deployed three EMC-Isilon parallel storage clusters, with a combined capacity of 1.5 petabytes. These clusters accommodate a variety of network filesystem protocols including NFS, Samba and iSCSI. Each modular, self-contained Isilon storage node contains a standalone fileserver with both hard disk and solid-state drives, processors, memory, and network interfaces. As additional nodes are added, all aspects of the cluster scale symmetrically, including capacity, throughput, memory, and fault tolerance. Each storage node is wired using dual 10 Gigabit Ethernet connections in order to provide maximum throughput to the computational resources.

The SAN hardware infrastructure is comprised of an SGI TP9400 & TP9500 storage arrays, Oracle 3510 & 7410 storage arrays, dual robotic tape silos, and a full complement of Brocade fibrechannel switches, providing 200 terabytes of fault-tolerant disk storage. Alternate paths exist throughout the fabric so that no single point of failure exists, guaranteeing access to critical data and processing power. The NRB facility utilizes two tape silos, a Storagetek SL8500 and a Quantum i6000 with a combined capacity of approximately 2.5 petabytes, to store mirrored copies of the facility's online, spinning-disk data. The i6000 tape silo is housed in the Reed data center, providing a secondary backup source and, owing to physical separation, comprising an element of a disaster recovery plan.

Additional Resources: The NRB Facility houses over 100 additional servers that provide the basis of its Enterprise-grade infrastructure. Basic services including DNS, DHCP, and SMTP relays are all deployed in redundant pairs. Authentication is handled using Active Directory, with performance, security, and failsafe best-practice considerations in mind. The facility also contains a ten-node VMWare vSphere cluster, allowing for rapid deployment of non-critical services as well as dedicated development and Q&A environments. The facility employs two Juniper hardware load balancers that split TCP/IP traffic between multiple, identical servers. Aside from load balancing generic network traffic, the units accelerate SSL communication, which is particularly important in transferring bulk amounts of sensitive research.

Postproduction Suite: The NRB Facility has a format and resolution-independent postproduction suite with A/V capture equipment and multiple video decks, including a Sony DSR 80 and a UVW 1800, capable of playing and recording in digital, component, Y/C and composite video. For audio, the suite has a Sony V77 sound processor, a 16-channel Mackie mixer, a 120-watt Crown amplifier and a JBL surround speaker package. In addition, the suite utilizes a 16-channel A/D Sierra router, a Miranda A/D converter and a DPS transcoder to facilitate video and audio signal routing. This equipment is connected to the Reality Monster described above for video and audio capture of real-time 3-D content processed by the supercomputer. Uncompressed content creation is done with AJA’s HD-capable digital disk recorder. To complement this hardware, the laboratory utilizes a variety of professional 3D and motion graphics packages, including Maya, Lightwave, and the full Adobe suite. The NRB Facility is also capable of virtual reality content creation to complement the visualization and stereoscopic capabilities of the DIVE, discussed below.

Network Resources: The NRB Facility intranet consists of 100baseT, Gigabit and 10 Gigabit Ethernet as well as an IEEE 802.11n compliant wireless network. Two Cisco Catalyst 3560G units and three

Enterprise-grade Cisco Catalyst 6500 Layer 3 switches provide redundant routing and non-blocking switching from the outside world to and from public-facing services, end-user workstations, and the network's core. A Juniper MX960, which is capable of handling 5.12 Terabytes/sec of traffic, connects the compute clusters to the Isilon storage, separating them as much as possible the rest of the network in order to reduce latency and increase throughput. The facility's routers and twenty Juniper switches use the Open Short Path First protocol to provide fast and fault-tolerant routing. System configurations for networking devices, as well as kernel-level parameters for Linux clients, are highly tuned to provide near line-level rates.

The facility is connected to the Cenic backbone of Internet2 via dual fiber Gigabit lines and utilizes a Cisco Firewall Services Module for edge network security. Both software-based PPTP and hardware-based SSL Virtual Private Network communications are provided for remote productivity and collaboration via encrypted communication.

Data Immersive Visualization Environment (DIVE): The DIVE features a 12' 150-degree floor-to-ceiling curved screen, on which real-time computer graphics, high definition video, stereoscopic 3D visualizations, or even simple slideshows are projected. The space provides investigators with the unique ability to visually "step inside" their data and analyze it in new ways. The projection system of the DIVE is comprised of a spherical hard-shell screen and three ceiling-mounted 3-chip DLP active stereo capable projectors, each rated at 5000 ANSI Lumens with a native 1280X1024 SXGA resolution. The projectors support optical blending of 12.5%, digital image warping and CLO, or constant light option, to ensure that image quality is maintained throughout the array. Two visualization workstations, each with dual NVIDIA graphics cards, drive these projectors, producing a 3840X1024 immersive display. A sophisticated A/V matrix along with an AMX remote control system facilitate audio and video routing from the DIVE to the conference room or other displays throughout the facility.

Magnetic Resonance Imaging Core Laboratory:

The Bruker Biospin 7.0 Tesla 30 cm clear bore MRI/MRS system is located in a 535 square foot space in the UCLA Ahmanson-Lovelace Brain Mapping Center. Three gradient systems are available: 1) a 200 mm inner diameter with a maximum gradient strength of 200 mT/m; 2) a 116 mm inner diameter with a maximum gradient strength of 400 mT/m, and 3) a 60 mm inner diameter with a maximum gradient strength of 950 mT/m. A variety of radio frequency volume coils and surface coils are available for use with these gradient systems, including three birdcage transmit/receive coils with inner diameters of 18mm, 35mm and 72mm. The instrument is capable of the full spectrum of modern neuroimaging including structural MRI, functional MRI, perfusion MRI, diffusion tensor MRI, and multinuclear MR spectroscopy. While the instrument is optimized for neuroimaging studies of rodents, it is also capable of imaging other body areas in rodents, including the heart and visceral organs. Imaging of postmortem tissue samples is also feasible. Full physiological monitoring is available including core temperature control and monitoring of heart and ventilation rate, end-tidal PCO2 and (non-invasive) blood pressure. A surgical suite, which includes a surgical microscope and a downdraft air exhaust table, is located in the adjoining room. The surgery and magnet rooms are equipped with isoflurane gas anesthesia equipment. A dedicated Linux workstation operating the Bruker Paravision software is available for offline image and spectroscopy processing. The 7.0 T 30 cm MRI Laboratory is available for use by UCLA-affiliated investigators who have research studies that require imaging of small animals, various phantoms and postmortem tissues. Operation and maintenance costs are recovered through user fees. From 8 AM to 6 PM, user fees are $200/hour. From 6 PM to 8 AM, user fees are the lesser of $125/hour (for scans 4 hours or less) or a capped $500 total overnight charge for up to 14 hours.

Description of Institutional Environment

UCLA, which ranks among the nation's top research universities, provides an excellent environment for all aspects related of the proposed research. The university is organized into a hierarchy of centers, institutes, departments and laboratories, each of which offers unique facilities that together form a strong network of research, educational and collaborative opportunities to provide an outstanding environment for career development and advancement. State-of-the art image acquisition and analysis facilities render UCLA particularly suited for career enhancement in the field of neuroimaging.

The Center for the Health Sciences at UCLA provides the most advanced medical technologies and cutting-edge research programs available. The David Geffen School of Medicine at UCLA is ranked ninth in the

country in NIH research funding and third for research dollars from all sources. The Brain Research Institute (BRI) is another organization that spans 26 different academic departments at UCLA. This institute serves to unite the highly diverse UCLA neuroscience community by initiating and fostering interdepartmental cooperation in research and education. The BRI's mission is to increase understanding of how the brain works, how it develops and how it responds to experience, injury and disease using multidisciplinary efforts to understand the nervous system at multiple levels with diverse technologies. In addition to the many training and collaborative opportunities provided by the BRI, which will be available to me through my position at the Department of Neurology, the BRI and the Neuropsychiatric Institute (NPI) offer the NPI Grand Rounds and the Joint Seminars in Neuroscience weekly. Internationally renowned speakers in the field are invited to present at these events.

The Department of Neurology within the David Geffen School of Medicine at UCLA possesses its own well-established research programs. These programs cover brain mapping and neuroimaging, and many other research agendas pertaining to neurological, neurosurgical and psychiatric populations. This department has been ranked #1 or #2 in NIH funding since 2002 and is also richly endowed with philanthropic donations. The Department of Neurology maintains strong collaborative ties with the NPI and most neurology faculty are also members of the BRI. Importantly, the Department of Neurology houses the ALBMC, which will provide the important facilities for this proposal as described above. Intellectual interactions and technical expertise are widely available in this laboratory environment, which includes several other key faculty members and a large technical and administrative staff.

Research at the Ahmanson-Lovelace Brain Mapping Center is also particularly relevant to the research and career goals described in this proposal. Research programs include refining and validating tools to integrate neuroscientific information across methods, populations, laboratories and species to promote a unified and standardized model of the working brain. This center provides training to students and colleagues, as befits a program within the School of Medicine, to better understand human neurological, neurosurgical and/or psychiatric disorders. A weekly seminar is provided that presents research related to these topics in an informal setting. In the past, I have presented at this seminar and with the opportunity to do so in the future, will benefit from the valuable feedback provided by Brain Mapping faculty, students and collaborators. The Brain Mapping Center is an outstanding environment for promoting career development in the field of neuroimaging research. As described above, it is among the most sophisticated image acquisition and image analysis neuroimaging laboratories in the country. Scanner access, computer resources, network resources and expertise will be readily available to me in this environment.

Overall, UCLA offers a fertile and productive research environment for established as well as junior scientists. The facilities and resources at UCLA are world class with several libraries and extensive computing facilities. The training opportunities are vast, with many focused programs in different departments that are accessible to developing scientists such as myself. Few other locations in the world offer the exceptional computer science-based image analysis technologies and imaging facilities of the ALBMC. In short, there is no better place to complete this proposal.

MAJOR EQUIPMENT

University of California, Los Angeles
As a premiere research institution, UCLA has cutting-edge facilities and technologies available for use by faculty and staff named in this application. Access to these facilities is greatly facilitated by the highly interactive and collaborative culture at our institution.

UCLA Core facilities, all of which are readily available for this proposal:

UCLA Informatics Center for Neurogenetics and Neurogenomics (ICNN)

UCLA Department of Medicine Statistics Core (DOMStat)

UCLA Semel Institute Biostatistics Core (SIStat)

UCLA Brain Mapping Core for Neuroimaging

Mitochondrial function / Synaptosome Core

Other within UCLA:

Microscopy and morphometric equipment: One Zeiss Axioplan II photomicroscope equipped for brightfield, differential interference contrast (DIC), and fluorescence illumination, with digital photography and storage (Axiovision), and computer controlled stage (x and y axis) and computer controlled z axis. Morphometric image analysis systems, Stereo Investigator and Neurolucida by MicroBrightfield Inc.. Consortium member with unlimited access to a Zeiss Scanning Confocal Laser Microscope
Tissue culture and general lab equipment: 3 tissue culture hoods, 2 incubators, liquid nitrogen storage tanks, Betaplate, Leica vibroslicer, PCR machine, Sorval centrifuge, Beckman ultracentrifuge, spectrophotometer, Revco Ultralow freezer, incubators, Olympus spin disc confocal with motorized stage, and Olympus fluorescence microscopes, a cryostat for tissue sectioning. Core facilities include a dark room for film development. The Neurobiology Core facility has an electron microscope. The UCLA Flow Cytometry core Facility is located across the street from RB1. An irradiator on the B floor of CHS is also available with an annual charge. Core Real Time PCR facilities are located in the Gonda building, adjacent to NRB1.
Surgical equipment: Two Zeiss operating microscopes with floor boom stands, video cameras and monitors. Two Kopf small animal stereotaxic devices with adapters for mouse. Two gas (isoflurane) anesthesia devices of veterinary grade quality with adapters for mouse and use with stereotaxic equipment. A comprehensive supply of high quality microsurgical instruments. Small autoclave for instruments.
In the Life Science Building: slide scanner, high quality color printers, machine shop and technical support in electronics. Leica RMXA compound brightfield/darkfield fluorescence microscope and color video camera, Leica dissection microscope, cryostat and rotary and sledge microtomes; spectrophotometer; two Zeiss operation (dissecting) microscopes; laminar flow hood; regulated CO_2 and low-oxygen incubators for tissue culture; autoclave; inverted phase microscope, Beckmann CS15-R refrigerated centrifuge, four thermal cyclers, ABI7300 real time PCR machine, shaker baths, incubators, hybridization ovens, gel documentation system, Speedvac. Behavioral testing apparatus: Accuscan hotplate, open field apparatus, elevated plus maze, water baths.

UCLA Intellectual and Developmental Disabilities Research Center (IDDC) Core services in confocal microscopy, image analysis. Brain Research Institute (BRI) core services in histology, electron microscope access, confocal microscopy and imaging.

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*(Commentary in same issue of *Lancet Neurology,* 15:789-791, 2016.

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Killestein, J., Schoonheim, M.M., **Voskuhl, R.R**. B-Cell Depletion and COVID-19 Severity in Multiple Sclerosis: Remaining Challenges. *Neurology*, 97:885-886, DOI 10.1212/WNL.0000000000012754, 2021.

Many publications are in journals with high impact factors: Nature, Nature Immunology, Lancet Neurology, Journal of Clinical Investigation (JCI), Brain, Proceedings of the National Academy of Sciences (PNAS) and JAMA Neurology. The breadth of interest to the scientific community is shown by publications beyond MS focused journals, namely those with very broad leadership interest (Nature, JCI, PNAS, Biology of Sex Differences), as well as those that are prestigious within the major disciplines of neurology and immunology (Lancet Neurology, JAMA Neurology, Nature Immunology). Underscoring the importance of specific contributions, four papers were highlighted by journal editors with invited commentaries and editorials about our papers (Lancet Neurology 2016, JAMA Neurology 2016, PNAS 2019, JCI 2019). The real world impact of my publications in the field of sex differences research has been demonstrated by my being interviewed four times by National Public Radio (NPR): Broadcasts on 6/2/14, 2/20/16, 11/24/17, 1/2/19, with yet another “Unsilencing” by Radiolab WNYC to broadcast on 8/19/21. I have done numerous interviews for media publications, including two for Neurology Today in 2020 and another in Nature Outlook Magazine with a feature story on sex differences in autoimmune diseases “The importance of sex”, July 15, 2021. I was also featured on FOX NEWS LA on May 18, 2022. In the landmark paper by Janine Clayton and Francis Collins in Nature regarding the establishment of the NIH policy to include Sex as a Biologic Variable (SABV) in NIH grants, one fifth of the literature cited was from my lab. My work in the field of sex differences research was recognized in the international community by my being awarded the Berlin Institute of Health (BIH) Excellence Award for Sex and Gender Aspects in Health Research in 2018, and by my being elected as President of the Organization for the Study of Sex Differences (OSSD) for the 2020-2022 term. My work in identifying neurodegenerative targets for disability-specific treatments in MS was recognized by my being selected for the Kenneth P. Johnson Memorial Lecture by Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS) in 2019. Moving from basic research discovery to translation toward novel clinic therapeutics, my work on novel estrogens and estrogen receptor (ER) ligands to treat neurodegenerative and autoimmune diseases was selected for a UCLA Innovation Award, in the UCLA campus wide competition sponsored by the UCLA Technology Development Group (TDG) in 2018. Underscoring translational potential, I am an inventor on over 10 UCLA patents. The estriol patent group is licensed by a new startup company founded on this technology, CleopatraRX (Houston, TX, USA, April 2020), while the ER beta ligand patent group is licensed by an established company, YuYu Pharmaceuticals (Seoul, South Korea, November 2020).

Lastly, I have presented as an invited speaker at numerous scientific meetings about our research publications, since January 1, 2021 this includes (but is not limited to): Americas Committee for Treatments and Research in Multiple Sclerosis (ACTRIMS) 2/25/21; Organization for the Study of Sex Differences (OSSD) 5/3/21; ACTRIMS Young Investigator Annual Meeting 5/28/21; Federation of Clinical Immunology Societies (FOCIS) 6/8/21; MSXchange / Montreal 11/5/21; International Society of Neuroimmunology (ISNI) 11/10/21; Nancy Davis Race to Erase MS 11/12/21; Alberta MS Network Seminar Series 1/20/22; Neuroimmunology Conferences of Stanford University 4/18/22; ACTRIMS Young Scientist Annual Meeting 4/20/22; Organization for the Study of Sex Differences (OSSD) 5/3/22; Ohio State Univ. Grand Rounds 5/17/22; American Aging Association Annual Meeting 5/20/22; Precision Medicine World Conference (PMWC2022) 6/30/22; Americas School of Neuroimmunology (ASNI) 7/12/22.

Zero papers have been retracted. The only Erratum in my career was misspelling of a middle author’s name in our Brain paper in 2018.

Mentoring, Service, and Inclusion

1) A description of commitment and dedication to mentorship and training in neuroscience research.

My track record which shows longstanding and recent commitment and dedication to mentorship and training in neuroscience (neuroimmunology and neurodegeneration) research:

At UCLA, I have mentored 12 postdoctoral fellows in my lab, 10 Ph.D. graduate students in my lab, 8 Ph.D. graduate students in other labs where I was co-mentor or a member on their PhD committees, 11 graduate students in my lab in non-Ph.D. programs, 67 UCLA undergraduates in my lab (with an average of 3 years each, with several working full time in one gap year after graduation), 2 nonUCLA undergraduates in my lab in summers, and 8 high school students in my lab in summers (often for 2 summers each). There would have been even more, but in March 2020 undergraduates and high school students were not allowed in labs at UCLA due to COVID pandemic safety rules. That said, graduate students and postdocs were allowed to be in the lab at different locations and times, with other work done remotely. Fortunately, all levels of trainees have fully returned to the lab, with masks, vaccinations, and COVID testing. I have also served as a faculty mentor to 7 junior faculty. As the UCLA MS Program Director, I recently envisioned and captured donor funding, led a national recruitment search, and worked with the UCLA Dept of Neurology Chair and West Los Angeles Veteran's Administration Chair to hire three MS junior faculty since 2018. All three were recruited from elite institutions (Harvard, Stanford, Penn).

I have worked with my research trainees to develop their careers. My trainees have received 50 awards, including 4 NIH F31 NRSA awards for graduate students and 4 National MS Society Postdoctoral Fellowships, a National Science Foundation Fellowship to teach science classes in predominantly Latin American high school and elementary schools, 19 training awards on the UCLA Laboratory of Neuroendocrinology NIH training grants(T32 HD007228), and 7 training awards on the UCLA Brain Research Institute (BRI) training grant. Over 90% of my peer reviewed research articles have had at least one trainee as a co-author. My postdoctoral fellows and graduate students have received numerous travel awards to present at meetings and won best poster awards at international, national, and UCLA competitions. These awards, papers, and presentations have propelled their careers across the spectrum: high school students getting accepted to top ranked universities; undergraduates getting accepted to Ph.D. programs, medical schools, and M.D., Ph.D. programs; graduate students getting desired postdoctoral fellowships; postdoctoral fellows getting positions as academic faculty or in the pharmaceutical industry; and junior faculty getting independent funding and tenure (Associate Professor). Space limitations do not allow listing of each.

In addition, I have given lectures to UCLA Dept of Neurology residents each year for over two decades and have been a formal lecturer in two UCLA classes each year in "Controversies in Clinical Trials" 2011-2020 and in "Neuroendocrinology of Reproduction", 2015-2020. I have participated in numerous CME courses, one of which was the NIH/ORWH/FDA sponsored Sex and Gender Course in Immunology (in 2019). This is a guide to teach researchers how to study sex as a biologic variable (SABV).

2) A description of my ongoing and planned outreach and mentoring activities, to enhance workforce diversity in the applicant's laboratory NOT-OD-20-031.

Track record in promoting diversity:

My background complies with NOT-NS-21-049 "NIH Research Grant (RO1) Applications from Individuals from Diverse Backgrounds, Including Under-Represented Minorities." I meet criteria in three categories:

1. Being female.
2. Growing up in the small rural town of Hennessey, Oklahoma (around 2,000 people) with a zip code (73742) that is listed by NIH as a low socio-economic region.
3. My mother was from eastern Oklahoma (Okemah) and was part Native American.

Leadership in advancing diversity:

1. As President of the Organization for the Study of Sex Differences, I established a new committee "Equity, Diversity, and Inclusion (EDI)", 2020.
2. ACTRIMS Annual Young Scientist Forum, Panelist on Advancing Diversity, 2020-present.
3. Laboratory of Neuroendocrinology, Lecturer Summer Series for Minority Students, 2011-2013.

Publications in advancing diversity:

1. Klein, R. S., Voskuhl, R., Segal, B. M., et al. Speaking out about gender imbalance in invited speakers improves diversity. ***Nature Immunology***, 18:475-478, 2017.

2. Voskuhl, R., Klein, S. Sex is a biological variable - in the brain too, ***Nature***, 568:171, 2019

Mentoring Minorities: Women as minorities in science.

1. Over 70% of all postdoctoral fellows, graduate students, undergrads, and high school students mentored by me have been female. My current graduate student is female (Ashley Loureiro, 2022-present), with yet another prospective female graduate student doing a rotation in the fall 2022 quarter. My other current graduate student is a Latin American male (Diego Cortez-Delgado).
2. I was chosen by an All Girls high school (Marymount High School) in Los Angeles to serve as a mentor and role model for women in leadership roles in the STEM field. I worked with Marymount to pioneer their summer internship program for high school girls from 2012-2017. The “SAILL Internship Program” grew exponentially in the first 5 years after its conception, starting with 2 girls per summer and expanding over the years to approximately 50 per summer. This introduces high school girls to STEM fields through experience in research labs, clinics and other professional work settings. It is now Marymount High School’s flagship program.

Mentoring Minorities: Ethnic minorities in science:

1. Laurie Morales, undergraduate independent research project, plus worked as lab asst. for a gap year before medical school. She was coauthor on several papers, one as first author in the *Proceedings of the National Academy of Sciences* (2006), functioning at the level of a graduate student, (2002-2006), Latin American.
2. Francisco Sandoval, undergraduate independent research project and worked as lab asst. for a gap year before going to a Master’s Program, (2007-2011). He obtained a Minority Biomedical Research Fellowship – Research Initiative for Scientific Enhancement (2011), Latin American.
3. Marina Ollervides-Ziehn, graduate student in Voskuhl lab (2008-2012). She received an NIH training grant fellowship from LNE (2009-2010), and was funded by the National Science Foundation to teach science classes in predominantly Latin American high school and elementary schools (2011-2012). She received her Ph.D. in in 2012, Latin American.
4. Rory Spence, graduate student in Voskuhl lab (2009-2013). He received NIH training grant fellowships from MCIP (2008-2011) and LNE (2011-2013), received Ph.D in 2013, Latin American.
5. Darian Mangu, undergraduate independent research project, (2015-2018), African American.
6. Emma Difippo, undergraduate independent research project, (2013-2015), Latin American.
7. Kevin Herrera, undergraduate independent research project, (2016-2018), Latin American.
8. Michelle Rivera, undergraduate independent research project, (2020-present) Latin American.
9. Diego Cortez-Delgado, graduate student, (2021-present) Latin American.

Outreach and mentoring activities to enhance diversity:

I hold leadership positions in two international organizations which focus on advancing diversity.

First, as President of the Organization for the Study of Sex Differences (OSSD), I established a new committee in 2020, the Equity, Diversity, and Inclusion (EDI) Committee. I tasked this committee with insuring EDI in all OSSD activities. The EDI has input during monthly executive meetings regarding how to enhance diversity via 1) the Program Committee so that diversity characterizes our annual meeting, 2) the Awards Committee so that award recipients include those of diverse backgrounds. At OSSD’s annual meeting in May 2021, my first annual meeting as President, more awards were presented to under-represented minorities (URM) than ever before, 3) the Nominations Committee increased the number of URM that run for OSSD Officer positions, and 4) the Membership & Communications Committee increased diversity in OSSD membership through social media. Our 2022 annual meeting was the most diverse to date. Thus, I have raised awareness of EDI in all OSSD activities. Our Jobs Board is where OSSD members reach out to a diverse applicant pool to hire, and URM candidates can post their CVs to potential OSSD faculty. OSSD studies sex differences due to sex hormones and sex chromosomes, as well as social and environmental factors. OSSD welcomes the LGBTQ+ community to its activities as we pursue complex issues related to sex and gender.

The second leadership position is with Americas Committee for Research and Treatment in MS (ACTRIMS) Annual Young Scientist Summit, as a Panelist on Advancing Diversity. As a faculty member on this panel each year, I get to meet and listen to URM trainees as they navigate the challenges they encounter. Being together and sharing experiences and ideas provides connections and a support system. Possible management solutions to problems they encounter are discussed leveraging how other’s experiences. Working with this group will also hopefully attract more URM trainees to my lab.

3) A description of planned activities during the grant period.

Promoting equitable scientific environments: I have and will continue to serve on UCLA graduate school admissions interview panels, taking into account the desire for a diverse class. I will draw attention to

performance by some that is exceptional in light of their disadvantaged background. I will continue to Chair and be a Member of panels for faculty recruitment and retention, as well as for selection of endowed chairs at UCLA. I will continue to mentor junior faculty, postdoctoral fellows, graduate students, undergraduates, and high school students, often either females, other under-represented minorities, or economically disadvantaged backgrounds, so they can reach their full potential. This will include encouraging them to apply for awards and submit posters and standing presentations at national and international meetings. When they succeed, it is not only for them, but also for others who identify with them. I will continue to co-author publications to advance diversity in selection of speakers for scientific meetings, and I will continue to serve on panels that select who speaks at meetings, to insure balance and diversity. I will continue to take advantage of support programs and diversity training offered by UCLA's EDI Office. Training offers time to reflect on one's own unintentional bias and increases awareness of impacts of our actions on others. I have often looked around the room at our lab meetings and been truly surprised at how diverse we are. Backgrounds are so different, yet these cultural differences fade, becoming invisible in the shared enthusiasm for the scientific discussion. It seems like such a "small world after all", not only at Disneyland Los Angeles, but also in my lab.

4) A description of significant contributions of service to the research community (e.g. study section service).

My track record of service to the research community:
National MS Society (NMSS), ad hoc reviewer of pilot grants, 1996-2010.
National Institutes of Health (NIH) Task Force on Women's Health 1997.
National MS Society Task Force on Gender and Autoimmunity 1997.
National Institutes of Health Study Section, ad hoc reviewer (BDCN), 1999-2002.
National MS Society Advisory Committee on Fellowships 2000-2002.
International ad hoc reviewer for French MS Society, 2002-present; Australian MS Society, 2003-present. Dutch MS Society, 2013, Ireland MS Society Medical Research Council, UK, 2017.
National Institutes of Health Study Section, chartered member and ad hoc reviewer (BDCN-4), 2002-2006.
National Multiple Sclerosis Society Scientific Advisory Peer Reviewer, Study Section A, 2007-2011.
Federation of Clinical Immunology Societies (FOCIS) Steering Committee, 2007-2008.
American Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS) Steering Committee, 2007-2012.
Scientific Review Committee for the joint meeting of the American (ACTRIMS), European (ECTRIMS) and Latin American (LACTRIMS) Programs, 2008.
Immune Tolerance Network (ITN) Steering Committee, 2009-2011.
National Institutes of Health Study Section, ad hoc reviewer, (NSD-C), 2009-2012.
Congressionally Directed Medical Research Program, Integration Panel, Department of Defense, 2013-2014.
National Institutes of Health Study Section NSD-C / NSD-A, ad hoc, 2013-2017
Organizing Committee, Organization for the Study of Sex Differences (OSSD), 2016-2018.
National Institutes of Health Study Section Ad hoc: HAI, 2018-2019.
National Institutes of Health Study Section ZNS1 SRB-H 12: R35 NIH grant reviews, 2019.
National Institutes of Health grant review: Sex and gender RFA: Special Emphasis Panel ZRG-1 The Intersection of Sex and Gender Influences on Health and Disease. 2020.
National Institutes of Health Study Section, ad hoc reviewer, CNBT, 2021.
National Institutes of Health Special Emphasis Panel review of Program Project grants, NIAID, 2022.
ACTRIMS, Advisory Committee, 2015-present.
ACTRIMS Annual Resident Summit (abstract reviewer and panelist), 2017-present;
ACTRIMS Annual Young Scientist Forum (invited speaker future MS therapies), 2018-present.
Nancy Davis Center Without Walls: Vision Setting & Patient Forum Panels UCLA site PI, 2019-present.
Department of Defense (DOD) Congressionally Directed Medical Research Program's (CDMRP) Multiple Sclerosis Research Program (MSRP), Programmatic Panel, 2020-present.
Organization for the Study of Sex Differences (OSSD), 6 year commitment: President-Elect, 2018-2020; President, 2020-2022; Past-President, 2022-2024.
Deutsche Forschungsgemeinschaft (DFG), German Federal Government for Excellence Cluster Initiatives, Clusters of Excellence Advisory Board Member, 2021-present.
Americas School of Neuroimmunology (ASNI), Faculty, 2022; International Society of Neuroimmunology (ISNI) Invited Speaker 2021.

Foreign Justification

Our collaborator Dr. Friedemann Paul of Univ. of Berlin, Germany transferred an existing database of multiple sclerosis and match healthy controls' clinical and imaging data to UCLA over a year ago. We will use the database that we now have in place at UCLA to conduct the research as described in the proposal (see Dr. Paul Letter of Support). This represents a scientifically useful dataset that Dr. Paul was willing to share with the UCLA team at no cost given that our scientific interests align with finding region-specific and sex-specific effects in multiple sclerosis. Dr. Paul is fully funded by German government, and as such no salary is requested from this proposal.

The collaboration between UCLA and Univ of Berlin began in the form a joint grant from the National Multiple Sclerosis Society (U.S.A.). The grant was to Univ. of Berlin with UCLA a subcontract. While the pilot funding ended a few years ago, collaboration between the two academic teams continued as a publication (Voskuhl, R.R., Patel, K., Paul, F., Gold, S.M., Scheel, M., Kuchling, J., Cooper, G., Asseyer, S., Chien, C., Brandt, A.U., Meyer, C.E., MacKenzie-Graham, A. Sex Differences in Brain Atrophy in Multiple Sclerosis. *Biology of Sex Differences*, 11(1):49. PMID: PMC7456053, 2020).

Here we are continuing to leverage this valuable resource and scientific collaboration in light of mutual research goals through further use of the larger, longitudinal database as described herein.

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Rhonda Middle Name R. Last Name*: Voskuhl Suffix: MD

Position/Title*:	Professor
Organization Name*:	The Regents of the University of California, Los Angeles
Department:	Neurology
Division:	David Geffen School of Medicine
Street1*:	635 Charles E. Young Drive South
Street2:	475D Neuroscience Research Bldg 1
City*:	Los Angeles
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Zip / Postal Code*:	90095-7334

Phone Number*: (310) 206-4636 Fax Number: (310) 206-9801

E-Mail*: rvoskuhl@ucla.edu

Credential, e.g., agency login: VOSKUHL2

Project Role*: PD/PI Other Project Role Category:

Degree Type: MD Degree Year: 1986

Attach Biographical Sketch*: File Name: R35_Biosketch_Voskuhl1071243835.pdf

Attach Current & Pending Support: File Name: NIH_OS_Voskuhl1071122820.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Yuichiro Middle Name Last Name*: Itoh Suffix: PhD

Position/Title*: Research Scientist
Organization Name*: The Regents of the University of California, Los Angeles
Department: Neurology
Division: David Geffen School of Med
Street1*: 710 Westwood Plaza
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City*: Los Angeles
County: Los Angeles County
State*: CA: California
Province:
Country*: USA: UNITED STATES
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Phone Number*: 310-825-9340 Fax Number:

E-Mail*: yito@mednet.ucla.edu

Credential, e.g., agency login: YUICHIROITO2

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2001

Attach Biographical Sketch*: File Name: Bioketch_Itoh1071123011.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Jin Middle Name Last Name*: Zhou Suffix: PhD

Position/Title*: Associate Professor
Organization Name*: The Regents of the University of California, Los Angeles
Department: Medicine
Division: GIM & HSR
Street1*: 1100 Glendon Avenue
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County: Los Angeles County
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Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90024-3503

Phone Number*: 310-794-3111 Fax Number:

E-Mail*: JinZhou@mednet.ucla.edu

Credential, e.g., agency login: jinjinzhou

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2011

Attach Biographical Sketch*: File Name: Biosketch_Zhou1071186260.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Allan Middle Name Last Name*: MacKenzie-Graham Suffix: PhD

Position/Title*: Associate Professor
Organization Name*: The Regents of the University of California, Los Angeles
Department: Neurology
Division: David Geffen School of Med
Street1*: 635 Charles E. Young Drive
Street2: NRB 225Z2
City*: Los Angeles
County: Los Angeles County
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90095-1769

Phone Number*: 310-267-5153 Fax Number: 310-206-7282

E-Mail*: amg@ucla.edu

Credential, e.g., agency login: MACKENZIEG2

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2006

Attach Biographical Sketch*: File Name: Biosketch_MacKenzie_Graham1071243205.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Prabha Middle Name Last Name*: Siddarth Suffix: PhD

Position/Title*:
Organization Name*: The Regents of the University of California, Los Angeles
Department: Semel Institute/Psychiatry
Division: Psychiatry
Street1*: 760 Westwood Plaza
Street2:
City*: Los Angeles
County: Los Angeles County
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90095-1759

Phone Number*: 310-825-4295 Fax Number:

E-Mail*: psiddarth@mednet.ucla.edu

Credential, e.g., agency login: siddarth2

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1988

Attach Biographical Sketch*: File Name: Biosketch_Siddarth1071186552.pdf

Attach Current & Pending Support: File Name:

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Rhonda R. Voskuhl, M.D.**

eRA COMMONS USER NAME (credential, e.g., agency login): VOSKUHL2

POSITION TITLE: **Professor of Neurology, Jack H. Skirball Chair in Multiple Sclerosis Research**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Phillips University, Enid, OK	BS	05/1982	Biology
Vanderbilt Medical School, Nashville, TN	MD	06/1986	Medicine
Univ. of Texas Southwestern, Dallas, TX	Resident	06/1990	Neurology
NIH, Neuroimmunology Branch, Bethesda, MD	Fellow	04/1995	Neuroimmunology

A. Personal Statement

I have devoted my career to translational research based on clinical observations in multiple sclerosis (MS) patients, from disentangling mechanisms in preclinical models to designing clinical trials of new treatments in a "Bedside to Bench to Bedside" approach. My lab was the first to use a cell-specific and region-specific transcriptomics approach to investigate the molecular basis for disability-specific disease progression in MS models. We also used MS postmortem tissues to validate genes identified in MS models. Collaboration with Dr. MacKenzie-Graham included neuroimaging of disability-specific regional gray matter atrophy in MS patients. I am an internationally recognized expert in sex differences research, demonstrating protective effects of estrogen and testosterone treatment in MS models, which I translated to 4 clinical trials in MS. At the lab bench, I was the first to show that estrogen receptor (ER) alpha and ER beta ligands were protective in EAE, acting through distinct mechanisms. I showed that ER beta ligation induced remyelination through direct action on oligodendrocytes, while downregulating innate immunity in central nervous system (CNS) by acting on microglia/macrophages, and that ER alpha ligation targeted astrocytes. I also discovered distinct roles for sex chromosomes in the immune system and the CNS in the MS model. Most recently, my lab began investigating the role of brain aging in worsening disability progression. My overarching mission is to use a region-specific, cell-specific, and sex-specific approach to identify novel treatment targets for females and males in MS preclinical models, then translate these findings to the clinic by designing trials to halt and repair of neurodegeneration, with enrollment optimally tailored for MS women and men.

My expertise in neuroimmunology, neuroscience, and sex differences is shown by comments/reviews:

Voskuhl, R. Itoh, Y. The X Factor in Neurodegeneration. **Journal of Experimental Medicine,** invited, minor revision, 2022.

Killestein, J., Schoonheim, M.M., Voskuhl, R., B-cell depletion and COVID-19 severity in MS. **Neurology,** 97:885, 2021.

Voskuhl, R. The effect of sex on MS risk and disease progression. **Multiple Sclerosis.** 26(554), 2020.

Voskuhl, R., A new cell subtype that confers neuroprotection. **Nature Immunology**, Nov. 3, 2020.

Voskuhl, R., Klein, S., Sex is a biological variable - in the brain too. **Nature**, 568 (7751):171, 2019.

Voskuhl, R., Wang, H., Elashoff, R. Why use sex hormones in relapsing-remitting multiple? **Lancet Neurology**, 15:790, 2016.

Voskuhl, R., Gold, S. Sex-related factors in multiple sclerosis susceptibility and progression. **Nature Reviews Neurology,** 8:255, 2012.

Ongoing projects and recently completed projects I would like to highlight:

NIH RO1NS109670 **Voskuhl (PI)** 12/01/18-11/30/23

Neuroprotection in MS: A Cell-specific and Region-specific Transcriptomics Approach

Goal: Understand neurodegenerative mechanisms in preclinical models of MS.

NIH RO1NS096748 **Voskuhl (PI)** 9/01/16-8/31/21

Parental Imprinting of the X Chromosome: Effects on Neurodegeneration

Goal: Determine effects of X imprinting on gene expression in the CNS during EAE.

The Conrad Hilton Foundation #18394 **Voskuhl (PI)** 7/1/19-6/30/22
Disability-specific disease modifying treatments (DMTs) in multiple sclerosis (MS)
Goal: Investigation of disability-specific treatments in EAE, MS, and brain aging.
NIH R21NS121806 **MacKenzie-Graham (PI)** 04/01/21-03/31/23
The Mechanism of Gray Matter Atrophy in Experimental Autoimmune Encephalomyelitis
Goal: To determine the role of oxidative stress in gray matter atrophy in EAE.
Role: Co-Investigator
NIH RO1NS112287 **Peter Clark (PI)** 7/1/19-6/30/24
Non-invasive Imaging of Brain Infiltrating T lymphocytes in a Mouse Model of EAE with PET
Goal: To image inflammation in vivo in EAE.
Role: Co-Investigator

My background complies with the NIH definition of an under-represented minority (NOT-NS-21-049). I meet criteria in three categories: 1) Being female, 2) Growing up in the small rural town of Hennessey, Oklahoma (around 2,000 people) with a zip code (73742) that is listed by NIH as a low socio-economic region, and 3) My mother was from eastern Oklahoma (Okemah) and was part Native American. Being a women from a rural, low income location has always been very challenging. I feel strongly that people in challenging circumstances should be supported. That is why I now serve on committees to enhance diversity, and I have a strong track record of recruiting women and URM in my lab as trainees. I worked with an All Girls High School (Marymount High School) for 5 years to start and nurture their mentorship program for “Girls in STEM”. It is very successful, and it is now their flagship program. Advancing the study of inclusion of women in science and research was one of the reasons why I become the President of the Organization for the Study of Sex Differences (OSSD). OSSD supports the development of women and URMs in career development as they aim to study the effect of sex on health and disease. Within months of becoming OSSD President in 2020, I started its Equality, Diversity, and Inclusion (EDI) committee. This committee is empowered to impact all aspects of OSSD activities: social media, officer nominations, annual meeting program, travel and poster awards, to name a few. In my recent tenure as OSSD President, I strengthened relationships with the Society for Women’s Health Research (SWHR) and the NIH Office of Research on Women’s Health (ORWH) so that our three organizations work closer together to advance our common goal of advancing inclusion of females in the study of sex as a biologic variable (SABV) in preclinical research and clinical research to promote precision therapeutic discoveries optimal for both women and men.

B. Positions and Honors

Positions and Employment

2004-present Professor, Dept. of Neurology, UCLA, Los Angeles, CA
2000-present Director, UCLA Multiple Sclerosis Program, UCLA, Los Angeles, CA
2000-2004 Associate Professor, Dept. of Neurology, UCLA, Los Angeles, CA
1995-2000 Scientific Director, UCLA Multiple Sclerosis Program, UCLA, Los Angeles, CA
1995-2000 Assistant Professor, Dept. of Neurology, UCLA, Los Angeles, CA
1994-1995 Senior Investigator, Neuroimmunology Branch, NIH, Bethesda, MD
1993-1994 Research Associate, Lab of Viral and Molecular Pathogenesis, NIH, Bethesda, MD
1990-1993 Clinical Associate, Neuroimmunology Branch, NIH, Bethesda, MD

Honors

2019-Kenneth P. Johnson Memorial Lecture, ACTRIMS annual meeting 2019
2018-Berlin Institute of Health (BIH) Excellence Award for Sex and Gender Aspects in Health Research
2018-UCLA Innovation Award, UCLA Campus-wide Technology Development Group Competition for 2018
2006-Jack H. Skirball Endowed Chair in MS Research
2001-California Congressman Henry Waxman Honorary Grant
1997-Harry Weaver Neuroscience Scholar of the National Multiple Sclerosis Society (NMSS)
1995-Outstanding Young Alumna, Phillips University
1994-Public Health Service Citation for Excellence in Research, National Institutes of Health (NIH)
1991-Annual Noble Lectureship Award
1988 and 1990-Texas Neurologic Society Annual Research Award for a Neurology Resident (twice)
1982-Oklahoma College All Star Women’s Basketball Team

1982-Overall Most Outstanding Senior Award, Phillips University
1979-1982-Biology Award (1979), Chemistry Award (1980), Science Award (1982), Phillips University
1978-1982-Four year full basketball scholarship - Two year Team Captain, Phillips University

Other Experience and Professional Memberships

Department of Defense (DOD) Congressionally Directed Medical Research Program's (CDMRP) Multiple Sclerosis Research Program (MSRP), Programmatic Panel, member, 2/6/2020-present.

Member, NIH Study Section BDCN, 2002-2006; NIH Special Emphasis Panel NSD-C, 2009-2012; NIH Study Section Ad hoc: NSD-C / NSD-A, 2013-2017; HAI, 2018-2019; CNBT, 2021; Special Emphasis Panel review of Program Project grants, NIAID, 2022.

Americas Committee for Treatments and Research in Multiple Sclerosis (ACTRIMS) Steering Committee, 2007-2012: ACTRIMS Advisory Committee, 2015-present; ACTRIMS Resident Summit, 2017-present; ACTRIMS Young Scientist Summit, 2018-present.

Presidential Leadership 6 year commitment to the Organization for the Study of Sex Differences (OSSD), President-Elect, 2018-2020; President, 2020-2022; Past-President, 2022-2024; OSSD Annual Meeting Organizing Committee, 2016-2018; NIH Office of Research on Women's Health (ORWH) / FDA Office of Women's Health Sex and Gender online course, 2020.

Deutsche Forschungsgemeinschaft (DFG), German Federal Government for Excellence Cluster Initiatives, Clusters of Excellence Advisory Board Member, 2021-present.

Americas School of Neuroimmunology (ASNI), Faculty, 2022; International Society of Neuroimmunology (ISNI) Invited Speaker 2021.

Lead Principle Investigator for UCLA, Nancy Davis Center Without Walls, Race to Erase MS, 2019-present.

C. Contributions to Science: Selected peer-reviewed publications.

1. Sex chromosome effects on autoimmunity and neurodegeneration. *I was the first to show sex differences in EAE using its relapsing-remitting model* (*Annals of Neurology,* 39:724-733, 1996). Over the last decade, my research has been discussed by others in *Nature* editorials three times regarding how sex differences can lead to insights into disease. I also am first author of an editorial on the subject (*Nature*, 568:171, 2019). My lab discovered sex chromosome effects in the immune system in both the MS and lupus models. We then identified a gene on the X chromosome (*Kdm6a*) that escapes X-inactivation in CD4+T lymphocytes as a mechanism for increased susceptibility of females to autoimmune disease. Also, we published that parental imprinting of the X chromosome can lead to sex differences in autoimmunity. Focusing on the CNS, we showed that in contrast to XX conferring an increase in autoimmunity, XY confers an increase in the neurodegenerative response to the same autoimmune attack. Indeed, *my lab was the first to show an effect of sex chromosome complement in the CNS in any neurodegenerative disease model* (in 2014). See below for special recognition, commentaries, and editorial highlights of publications in this field.

a. Smith-Bouvier, D.L., Divekar, A.A., Sasidhar, M., Du, S., Tiwari-Woodruff, S., King, J.K., Arnold, A.P., Voskuhl, R.R. **(**2008) A role for sex chromosome complement in the female bias in autoimmune disease. **Journal of Experimental Medicine,** 20205(5):1099-108, PMCID: PMC2373842.

b. Du, S., Itoh, I., Askarinam, S., Hill, H., Arnold, A., Voskuhl, R.R. (2014) XY Sex Chromosome Complement, Compared with XX, in the CNS Confers Greater Neurodegeneration During EAE. **Proceedings of the National Academy of Sciences (PNAS),** 111:2806-2811. PMCID: PMC3932937.
*(Recognized in ***Lancet Neurology*** as one of the top 5 in MS for 2014).

c. Itoh, Y., Golden, L., Itoh, N., Matsukawa, M., Ren, E., Tse, V., Arnold, A.P., Voskuhl, R.R. (2019) The X-linked histone demethylase *Kdm6a* in CD4+ T lymphocytes modulates autoimmunity. **Journal of Clinical Investigation (JCI),** https://doi.org/10.1172/JCI126250, 130:3852-3863, PMCID: PMC6715385.
*(Commentary on this article in **JCI** 130:3536-3538, 2019.)

d. Golden, L.C., Itoh, Y., Itoh, N, Iyengar, S., Coit, P., Salama, Y., Arnold, A.P., Sawalha AH, Voskuhl, R.R. (2019) Parent-of-origin differences in DNA methylation of X chromosome genes in T lymphocytes. **Proceedings of the National Academy of Sciences (PNAS),** PMCID: PMC6936674.
*(Editorial Highlight of this paper "In This Issue" section **PNAS**.)

2. Basic insights into neurodegeneration. These studies used a cell-specific and region-specific transcriptomics approach to identify novel mechanisms underlying regional neuropathology in MS models and MS autopsy tissues. While this approach had been used in astrocytes during health, *my lab was the first to use a cell-specific and region-specific transcriptomics approach in any neurodegenerative disease model* (in 2018).

a. Itoh,Y. Voskuhl, R.R. (2017) Cell specificity dictates similarities in gene expression in multiple sclerosis, Parkinson's disease, and Alzheimer's disease. **PLoS One**, 12:e0181349, PMCID 5513529.
b. Itoh, N., Itoh, Tassoni, A., Ren, E., Kaito, M., Ohno, A., Y., Ao, Y., Farkhondeh, V., Johnsonbaugh, H., Burda, J. Sofroniew, M.V., Voskuhl, R.R. (2018) Cell-Specific and Region-Specific Transcriptomics in the multiple sclerosis model: Focus on astrocytes. **Proceedings of the National Academy of Sciences (PNAS)**, 115:E302-E309, PMCID: PMC5777065.
c. Voskuhl, R.R., Itoh, N., Tassoni, A., Matsukawa, M., Ren, E., Tse, V., Jang, E., Suen, T., Itoh, Y. (2019) Gene expression in oligodendrocytes during remyelination reveals cholesterol homeostasis as a therapeutic target in multiple sclerosis. **Proceedings of the National Academy of Sciences (PNAS)**, 116 (20):1-130-10139, PMCID: PMC6525478.
d. Tassoni, A., Farkhondeh, V., Itoh, Y., Itoh, N., Sofroniew, M.V., Voskuhl, R.R. (2019) The astrocyte transcriptome in EAE optic neuritis shows complement activation and reveals a sex difference in astrocytic C3 expression. **Scientific Reports**, 9:10010-22, PMCID: PMC6620300.

3. Identified the cell that mediates estrogen's neuroprotective effect *in vivo* in the MS model. Estrogens were known to be neuroprotective through actions on estrogen receptors (ERs) for decades, however which cell in the CNS mediated this neuroprotection *in vivo* remained unknown. My lab created cell-specific knock outs of ER alpha and ER beta to determine which CNS cell mediated neuroprotection *in vivo*. *My lab was the first to identify which cell is responsible for estrogen mediated neuroprotection in vivo in any neurological disease model.*
a. Tiwari-Woodruff, S., Morales, L., Lee, R., Voskuhl, R.R. (2007) Differential Neuroprotective and Anti-inflammatory Effects of Estrogen Receptor (ER) α and ER β Ligand Treatment. **Proceedings of the National Academy of Sciences (PNAS),** 104:14813-14818, PMCID: PMC1976208.
b. Spence, R., Hamby, M., Umeda, E., Itoh, N., Du, S., Bondar, G., Lam, J., Ao, Y., Wisdom, A., Cao, Y., Sandoval, F., Sofroniew, M.V., Voskuhl, R.R. (2011) Neuroprotection mediated through estrogen receptor alpha on astrocytes. **Proceedings of the National Academy of Sciences (PNAS),** 108:8867-8872. PMCID: PMC3102368.
c. Spence, R.D., Wisdom, A.J., Cao, Y., Hill, H.M., Mongerson, C., Stapornkul, B., Itoh, N., Sofroniew, M.V., Voskuhl, R.R. (2013) Estrogen signaling through ER-alpha but not ER-beta on astrocytes mediates neuroprotection during EAE and decreases astrocyte levels of proinflammatory chemokines. **Journal of Neuroscience,** 33:10924-109333. PMCID: PMC3693061.
d. Kim, R., Hoffmann, A., Mangu, D. Kavosh, R., Jung, E., Itoh, N., Voskuhl, R.R. (2018) Estrogen Receptor Beta Ligand Acts on $CD11c^+$ Cells to Mediate Protection in Experimental Autoimmune Encephalomyelitis. **Brain**, 141:132-147. PMCID: PMC5837360.

4. Clinical trials. I have translated basic findings in my lab to four Phase 2 clinical trials in MS. First, I translated my lab's preclinical finding that the estrogen of pregnancy (estriol) is anti-inflammatory and neuroprotective. *This has implications for the mechanism underlying the protection of pregnancy in MS.* Translation entailed three clinical trials (2 multisite and 1 single site). The first estriol trial showed a reduction in enhancing lesions (*Annals of Neurology).* The 16 site estriol trial showed a reduction in relapses as powered as the primary outcome for a Phase 2 trial and an improvement in cognition as an exploratory (*Lancet Neurology* and featured by a Commentary). We then mapped estriol treatment induced sparing atrophy in cerebral cortex (*Brain & Behavior,* 2018), and showed an estriol treatment mediated reduction in serum neurofilament light chain (sNfL) levels (*Ann. Clin. & Trans, Neurol*., 2022). My lab was also the *first to show that testosterone treatment is protective in EAE* (*J. Immunology*, 159:3-6, 1997). We translated this to a pilot clinical trial in MS men (*Archives of Neurology* 64:683-688, 2007, aka *JAMA Neurology*), then mapped regions of testosterone mediated sparing of gray matter atrophy in MS men (*Neuroimage Clinical,* 4:454-460, 2014*).*
a. Sicotte, N., Liva, S.M., Klutch, R., Pfieffer, P., Bouvier, S., Odesa, S., Wu, T.C.J., Voskuhl, R.R. (2002) Treatment of multiple sclerosis with the pregnancy hormone estriol. **Annals of Neurology,** 52:421-428.
b. Voskuhl, R.R., Wang, H., and the Estriol Trial Study Group (2016) Estriol combined with glatiramer acetate for women with relapsing-remitting MS: A Randomised, Placebo-Controlled, Phase 2 Trial. **Lancet Neurology**, 15: 35-46. PMID 26621682.
*(Commentary: Voskuhl, R., Wang, H., Elashoff, R. **Lancet Neurology,** 2016, 15:790-791)
c. MacKenzie-Graham, A., Brook, J., Kurth, F., Itoh, Y., Meyer, C., Montag, M., Wang, H., Elashoff, R., Voskuhl, R.R. (2018) Estriol-mediated neuroprotection in multiple sclerosis localized by voxel-based morphometry. 8(9):e01086. **Brain & Behavior,** PMCID: PMC6160650.

d. Voskuhl, R., Kuhle J., Siddarth , P., Itoh, N., Patel, K., **MacKenzie-Graham, A**. (2022) Decreased Neurofilament Light Chain Levels In Estriol-Treated Multiple Sclerosis. **Annals of Clinical & Translational Neurology,** doi:10.1002/acn3.51622. PMID: 35770318.

5. Clinical Research. I initially studied immune responses in the peripheral blood of MS patients during the hormone treatment trials where I was the PI. Then I focused on the CNS. In collaboration with Dr. MacKenzie-Graham, *we mapped 3 different MS disabilities to distinct gray matter regions, which* was highlighted by an editorial in *JAMA Neurology*. *We also showed sex differences in regional gray matter atrophy in MS patients* by comparing gray matter atrophy in female MS vs female healthy controls and by comparing male MS vs male healthy controls (to remove the confound of sex differences in healthy brain).
a. Soldan, S.S., Alvarez-Retuerto, A.I., Sicotte, N.L., Voskuhl, R.R. (2003) Th1 to Th2 immune shift in female multiple sclerosis patients treated with the pregnancy hormone estriol, **Journal of Immunology,** 11:6267-6274.
b. Gold, S., Chalifoux, S., Giesser, B., Voskuhl, R.R. (2008) "Immune Modulation and Increased Neurotrophic Factor Production in Multiple Sclerosis Patients treated with Testosterone." **Journal of Neuroinflammation,** 5:32: 1-8. PMID: PMC2518142.
c. MacKenzie-Graham, A., Kurth, F., Itoh, Y., Wang, H., Montag, M., Elashoff, R., Voskuhl, R. (2016) Disability-Specific Atlases of Gray Matter Loss in Relapsing-Remitting Multiple Sclerosis. **JAMA Neurology**, 73:944-953, PMCID: 27294295
*(Editorial highlight in same issue of **JAMA Neurology, (**2016), 73(8):910-912.
d. Voskuhl, R.R., Patel, K., Paul, F., Gold, S.M., Scheel, M., Kuchling, J., Cooper, G., Asseyer, S., Chien, C., Brandt, A.U., Meyer, C.E., MacKenzie-Graham, A. (2020) Sex Differences in Brain Atrophy in Multiple Sclerosis. **Biology of Sex Differences**, 11(1):49. PMCID: PMC7456053.

See URL for list of publications:

https://pubmed.ncbi.nlm.nih.gov/?term=%28%28rhonda+voskuhl%5BAuthor%5D%29+OR+Voskuhl+RR%5BAuthor%5D%29+OR+voskuhl+r%5BAuthor%5D&sort=date

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Itoh, Yuichiro

eRA COMMONS USER NAME (credential, e.g., agency login): YUICHIROITO2

POSITION TITLE: Project Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Tohoku University, Sendai, Miyagi	BS	03/1996	Molecular Biology
Tohoku University, Sendai, Miyagi	PHD	03/2001	Molecular and Cell Biology
UCLA, Los Angeles, California	Postdoctoral Fellow	11/2007	

A. Personal Statement

I have over 24 years' experience in molecular biology and genetics. In the beginning, as a graduate student, I cloned novel sex chromosome linked genes in chicken, and contributed to development of concepts regarding avian sex chromosomes, which were relatively unstudied at that time. I used methods of cytology including karyotyping of metaphases, mapping of DNA and RNA probes to chromosomes, immunohistochemistry, gene cloning and manipulation. In Dr. Art Arnold's lab at UCLA, I discovered that in birds, sex chromosome dosage compensation is ineffective, an unexpected result based on previous work where dosage compensation of the X chromosome in mammals is quite effective. The major focus of the lab was to understand the role of sex chromosomes in brain development. In these studies and others, I have utilized bioinformatics and global analysis of the transcriptome. Over last several years, working with the Voskuhl team, I have led the RNA sequencing and bioinformatics analysis using a cell-specific and region-specific approach to discover disability specific neuroprotective treatments for multiple sclerosis (MS). To this end, we have several papers published using RiboTag technology showing gene expression changes in various parts of the CNS during health and disease, with my contribution of applying bioinformatics to study cell type specific transcriptomes. Also, we published a paper reporting the deleterious effect of *Kdm6a* gene in CD4+ T-cells in MS mouse model, which could explain the sex bias of autoimmune diseases. Thus, I am ideally suited for work in this proposal that utilizes RiboTag technology and bioinformatics, to determine cell-specific, region-specific, sex-specific, and age-specific molecular mechanisms in disease, to develop the tailored treatments in women and men with MS.

1. Itoh Y, Golden LC, Itoh N, Matsukawa MA, Ren E, Tse V, Arnold AP, Voskuhl RR. The X-linked histonedemethylase Kdm6a in CD4+ T lymphocytes modulates autoimmunity. J Clin Invest. 2019 Aug12;129(9):3852-3863. PubMed Central PMCID: PMC6715385.
2. Voskuhl RR, Itoh N, Tassoni A, Matsukawa MA, Ren E, Tse V, Jang E, Suen TT, Itoh Y. Geneexpression in oligodendrocytes during remyelination reveals cholesterol homeostasis as a therapeutictarget in multiple sclerosis. Proc Natl Acad Sci U S A. 2019 May 14;116(20):10130-10139. PubMedCentral PMCID: PMC6525478.
3. Itoh N, Itoh Y, Tassoni A, Ren E, Kaito M, Ohno A, Ao Y, Farkhondeh V, Johnsonbaugh H, Burda J,Sofroniew MV, Voskuhl RR. Cell-specific and region-specific transcriptomics in the multiple sclerosismodel: Focus on astrocytes. Proc Natl Acad Sci U S A. 2018 Jan 9;115(2):E302-E309. PubMed CentralPMCID: PMC5777065.

4. Itoh Y, Voskuhl RR. Cell specificity dictates similarities in gene expression in multiple sclerosis,Parkinson's disease, and Alzheimer's disease. PLoS One. 2017;12(7):e0181349. PubMed CentralPMCID: PMC5513529.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2019 – Project Scientist, UCLA, Los Angeles, CA
2010 – 2019 Assistant Researcher, UCLA, Los Angeles, CA
2007 – 2010 Staff Research Associate III, UCLA, Los Angeles, CA
2002 – 2007 Postdoctoral Fellow, UCLA, Los Angeles, CA
2001 – 2002 Postdoctoral Research Associate, Tohoku University

Honors

2002 – 2002 Yamada Life Science Foundation Award, Yamada Life Science Foundation
1998 – 2001 3 year stipend and partial research support, Japan Society for the Promotion of Science
1999 – 2000 Inui Memorial Foundation Award, Inui Memorial Foundation

C. Contributions to Science

1. <Sex difference of neurodegenerative disease> Using the cell specific conditional knock out mice, I showed the deleterious role of X-linked histone demethylase Kdm6a in CD4+ T-cells in MS mouse model, indicating the importance of this X escapee gene in the sexually dimorphism of autoimmune disease. In addition, transcriptome and methylome analysis showed the parental epigenetic difference in X chromosome.

a. Golden LC, Itoh Y, Itoh N, Iyengar S, Coit P, Salama Y, Arnold AP, Sawalha AH, Voskuhl RR. Parent-of-origin differences in DNA methylation of X chromosome genes in T lymphocytes. Proc Natl Acad Sci U S A. 2019 Dec 10; PubMed Central PMCID: PMC6936674.
b. Itoh Y, Golden LC, Itoh N, Matsukawa MA, Ren E, Tse V, Arnold AP, Voskuhl RR. The X-linked histone demethylase Kdm6a in CD4+ T lymphocytes modulates autoimmunity. J Clin Invest. 2019 Aug 12;129(9):3852-3863. PubMed Central PMCID: PMC6715385.
c. Voskuhl RR, Sawalha AH, Itoh Y. Sex chromosome contributions to sex differences in multiple sclerosis susceptibility and progression. Mult Scler. 2018 Jan;24(1):22-31. PubMed Central PMCID: PMC5823689.

2. <Neurodegenerative disease in mouse models> I carried out genetic studies to reveal mechanisms underlying neurodegeneration in the mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis. This entailed defining the transcriptome of astrocytes and doing bioinformatics pathway analyses in a cell-specific and region-specific manner.

a. Tassoni A, Farkhondeh V, Itoh Y, Itoh N, Sofroniew MV, Voskuhl RR. The astrocyte transcriptome in EAE optic neuritis shows complement activation and reveals a sex difference in astrocytic C3 expression. Sci Rep. 2019 Jul 10;9(1):10010. PubMed Central PMCID: PMC6620300.
b. Voskuhl RR, Itoh N, Tassoni A, Matsukawa MA, Ren E, Tse V, Jang E, Suen TT, Itoh Y. Gene expression in oligodendrocytes during remyelination reveals cholesterol homeostasis as a therapeutic target in multiple sclerosis. Proc Natl Acad Sci U S A. 2019 May 14;116(20):10130-10139. PubMed Central PMCID: PMC6525478.
c. Itoh N, Itoh Y, Tassoni A, Ren E, Kaito M, Ohno A, Ao Y, Farkhondeh V, Johnsonbaugh H, Burda J, Sofroniew MV, Voskuhl RR. Cell-specific and region-specific transcriptomics in the multiple sclerosis model: Focus on astrocytes. Proc Natl Acad Sci U S A. 2018 Jan 9;115(2):E302-E309. PubMed Central PMCID: PMC5777065.

3. <Neurodegenerative disease in humans> As a translational application of our basic discoveries, I applied a data mining approach to determine cell specific transcriptome features in human neurodegenerative diseases (multiple sclerosis, Alzheimer's Disease and Parkinson's Disease). I was also involved data analyses, including heatmap representations of clinical and MRI data in studies in multiple sclerosis patients.

a. MacKenzie-Graham A, Brook J, Kurth F, Itoh Y, Meyer C, Montag MJ, Wang HJ, Elashoff R, Voskuhl RR. Estriol-mediated neuroprotection in multiple sclerosis localized by voxel-based morphometry. Brain Behav. 2018 Sep;8(9):e01086. PubMed Central PMCID: PMC6160650.

b. Itoh Y, Voskuhl RR. Cell specificity dictates similarities in gene expression in multiple sclerosis, Parkinson's disease, and Alzheimer's disease. PLoS One. 2017;12(7):e0181349. PubMed Central PMCID: PMC5513529.

c. MacKenzie-Graham A, Kurth F, Itoh Y, Wang HJ, Montag MJ, Elashoff R, Voskuhl RR. Disability-Specific Atlases of Gray Matter Loss in Relapsing-Remitting Multiple Sclerosis. JAMA Neurol. 2016 Aug 1;73(8):944-53. PubMed Central PMCID: PMC6415681.

4. <Sex chromosome mouse models> To study sex difference, we have been using several different mouse models to separately analyze the sex chromosome, organizational, and activational factors. One of the major mouse model, four core genotypes, has Sry deletion from Y chromosome and Sry-transgene insertion on autosome. Although this model has been used in many different studies, its transgenic character had not been carefully investigated. We characterized this mouse model at the sequence level and reported. I also contributed in several studies utilizing sex chromosome mouse models, as a biostatistician and a cytogeneticist.

a. Ghosh MK, Chen KE, Dill-Garlow R, Ma LJ, Yonezawa T, Itoh Y, Rivera L, Radecki KC, Wu QP, Arnold AP, Muller HK, Walker AM. Sex Differences in the Immune System Become Evident in the Perinatal Period in the Four Core Genotypes Mouse. Front Endocrinol (Lausanne). 2021;12:582614. PubMed Central PMCID: PMC8191418.

b. Umar S, Cunningham CM, Itoh Y, Moazeni S, Vaillancourt M, Sarji S, Centala A, Arnold AP, Eghbali M. The Y Chromosome Plays a Protective Role in Experimental Hypoxic Pulmonary Hypertension. Am J Respir Crit Care Med. 2018 Apr 1;197(7):952-955. PubMed Central PMCID: PMC6020406.

c. Itoh Y, Arnold AP. Are females more variable than males in gene expression? Meta-analysis of microarray datasets. Biol Sex Differ. 2015;6:18. PubMed Central PMCID: PMC4640155.

d. Itoh Y, Mackie R, Kampf K, Domadia S, Brown JD, O'Neill R, Arnold AP. Four core genotypes mouse model: localization of the Sry transgene and bioassay for testicular hormone levels. BMC Res Notes. 2015 Mar 7;8:69. PubMed Central PMCID: PMC4354741.

5. <Sex chromosome studies in birds and mammals> In animals with heteromorphic sex chromosomes, dosage compensation of sex-chromosome genes is thought to be critical for species survival. Despite the molecular mechanism of X chromosome dosage compensation in mammals was well studied, the one in birds has not been understood. In birds, females (ZW) and males (ZZ) differ in the number of Z chromosomes. We published the first global analysis of chicken and zebra finch genes, and showed that Z chromosome dosage compensation is inefficient. On the other hand, in bovine blastocysts, X chromosome inactivation is not complete. We reported the global analysis of bovine blastocysts genes, showing that X genes regulate A (autosomal) genes. The zebra finch is often studied because of its interesting behavior and neurobiology. Unfortunately, genetic information on this species has been lacking. Using molecular and cytogenetic techniques, I characterized zebra finch chromosomes and found chromosomal polymorphisms and evolutionary arrangement. I also reported novel chicken and zebra finch W chromosome repetitive sequence which is the major component of W sequence. The information which I reported contributed to the zebra finch genome project. I also established two zebra finch cell lines to provide materials to the community.

a. Itoh Y, Arnold AP. X chromosome regulation of autosomal gene expression in bovine blastocysts. Chromosoma. 2014 Oct;123(5):481-9. PubMed Central PMCID: PMC4170013.

b. Itoh Y, Replogle K, Kim YH, Wade J, Clayton DF, Arnold AP. Sex bias and dosage compensation in the zebra finch versus chicken genomes: general and specialized patterns among birds. Genome Res. 2010 Apr;20(4):512-8. PubMed Central PMCID: PMC2847754.

c. Itoh Y, Melamed E, Yang X, Kampf K, Wang S, Yehya N, Van Nas A, Replogle K, Band MR, Clayton DF, Schadt EE, Lusis AJ, Arnold AP. Dosage compensation is less effective in birds than in mammals. J Biol. 2007;6(1):2. PubMed Central PMCID: PMC2373894.

d. Itoh Y, Mizuno S. Molecular and cytological characterization of SspI-family repetitive sequence on the chicken W chromosome. Chromosome Res. 2002;10(6):499-511. PubMed PMID: 12489831.

See URL at
https://www.ncbi.nlm.nih.gov/myncbi/yuichiro.itoh.1/bibliography/public

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Zhou, Jin

eRA COMMONS USER NAME (credential, e.g., agency login): JINJINZHOU

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Nanjing Normal University, Nanjing, China	BA	06/2002	Mathematics
Nankai University, Tianjin, China	MS	06/2005	Applied Mathematics
University of California, Los Angeles, CA	PhD	03/2011	Biomathematics
Harvard University, Boston, MA	Postdoctoral Fellowship	08/2013	Biostatistics

A. Personal Statement

I am an Associate Professor at the Department of Medicine Statistics Core, UCLA Division of General Internal Medicine and Health Services Research. I was trained in mathematics, statistics, and genetics. My work on statistical genomics and biomedical informatics focuses on developing statistically powerful and computationally efficient tools for biobank scale genetic association studies, metagenomics data analysis, and personalized treatment prediction using electronic medical records (EHRs) data. My career goal is to be at the interface of statistics, genetics, and biomedical informatics and better utilize “big” health-related data for personalized healthcare. I have extensive expertise in dissecting the genetic components of complex diseases. Besides methodological development, I have broad collaboration experiences. I have collaborated with clinicians and epidemiologists at the University of Arizona and Channing's Laboratory at Brigham and Women's Hospital (BWH) for their bulk and single cell transcriptomics studies. I am also an active contributor of Million Veteran Program (MVP). My roles range from study design to developing the detailed analysis plan. My experience is ideally suited to my role in the proposed research in this R35 which will involve creation of a transcriptomics database from central nervous system tissues of RiboTag mice from 4 specific cell types, multiple brain regions, two sexes, and three ages to do metagenomics data analyses to identify cell-specific gene expression targets tailored by disability, sex, and age.

Ongoing and recently completed projects that I would like to highlight include:

VA-Department of Energy Joint Research Concept
Role: Co-PI | PI: Reaven
07/01/2022 – 06/30/2024
Dynamic Prediction of Short-Term and Long-Term Diabetes Complications Leveraging Massive Electronic Health Records, Million Veteran Project, Machine Learning/Artificial Intelligence, and High-Performance Computing

NSF DMS-2054253
Role: MPI | Contact PI: H. Zhou
07/01/2021 - 06/30/2025
Statistical methods and computational algorithms for biobank data

NIH/NHLBI, R21 HL150374
Role: Zhou (Contact PI) | MPI: Reaven
08/01/2020-07/31/2023
A Role for Glycemic Variation in Optimizing Management of Diabetes and Vascular Complications

NIH/NHGRI, R01 HG006139
Role: Co-I | Subaward PI: Sobel
07/01/2020–06/30/2024
Genomics, EHRs, GPUs, and Next Generation Computational Statistics

ABRC, ADHS16-162409
Role: PI
03/01/2017-02/28/2021
Develop Data-Driven Precision T2D Treatment Regime using Veteran Healthcare Database

NIH/NIDDK, K01 DK106116
Role: PI
08/11/2016-08/31/2020 (NCE)
Develop T2D Patient-Centered Treatment Suggestion Rule Using EMR Data

Phoenix VA Health Care System, 015969-0001
10/15/2019-09/30/2020
PVAHCS IPA Agreement for Jin Zhou

NIH/NIDDK/NIEHS, R01 DK123113
Role: Co-I | PI: Zhao
09/01/2020-06/30/2025
Molecular Mechanism Underlying the Regulation of Manganese Homeostasis

NIH/NHLBI, R01 HL149744
09/01/2020-08/31/2025
Role: Co-I | Polverino
B Cell-Adaptive Immune Profile in Emphysema-Predominant COPD

Citations:

1. C. A. German, J. S. Sinsheimer, Y. C. Klimentidis, H. Zhou, and **J. J. Zhou**. (2020) Ordered multinomial regression for genetic association analysis of ordinal phenotypes at biobank scale. Genetic Epidemiology. 44(3): 248-260. PMID: 31879980
2.
3. Doubleday K, Zhou H, Fu H, **Zhou JJ**. An Algorithm for Generating Individualized Treatment Decision Trees and Random Forests. J Comput Graph Stat. 2018;27(4):849-860. 2018 Jun 14. PMID: 32523325; PMCID: PMC7286561.
4. M. Vujkovic, J. M. Keaton, J. A. Lynch, D. R. Miller, **J. J. Zhou**, et al. (2020) Discovery of 318 new risk loci for type 2 diabetes and related vascular outcomes among 1.4 million participants in a multi-ethnic meta-analysis, Nature Genetics, 52:680-691. PMID: 32541925 PMCID: PMC7343592

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021-Present	Associate Professor, UCLA Department of Medicine Statistics Core, Los Angeles, CA
2019-2021	Associate Professor, University of Arizona, College of Public Health, Tucson, AZ
2013-2019	Assistant Professor, University of Arizona, College of Public Health, Tucson, AZ
2015-Present	Membership Committee Member, Caucus Women in Statistics
2011-Present	Member, Eastern North American Region, International Biometric Society
2010-Present	Member, American Statistical Association
2009-Present	Member, American Society of Human Genetics

2009-Present Communication Committee Member, International Genetic Epidemiology Society

Honors

2012 Program in Quantitative Genomics Stellar Abstract Award, Harvard University
2012 Travel Award, 14th Meeting of New Researchers in Statistics and Probability
2012 Program in Quantitative Genomics Travel Award, Harvard University
2009 Dissertation Year Fellowship, University of California, Los Angeles

C. Contributions to Science

1. Development of statistical and computational tools. Large-scale and high-dimensional data are being generated at an unprecedented rate. In my 10 years of research, I have devoted myself to develop quantitative methods and software to facilitate using these large-scale datasets. In my previous research, I have addressed some challenges of utilizing familial data for gene mapping and made significant contributions to the field of genetic studies using family designs. Given the complexity of the design and scale of the genomic data, analysis tools either rely on simplistic assumptions or suffer from computational bottleneck. I have built regularization models for combinatorial process to improve the interpretability of these genomics data. Methods I developed reduced the computational time from days to hours with superior statistical power. Free user-friendly software packages were provided for each method I developed. My current research focuses on developing tools for metagenomics data and next generation sequencing (NGS) data.
    a. Hu L, Lv W, **Zhou JJ**, Zhou H. MM Algorithms for Variance Component Estimation and Selection in Logistic Linear Mixed Model. Stat Sin. 2019;29(3):1585-1605. PMCID: PMC7286582.
    b. Zhai J, Knox K, Twigg HL 3rd, Zhou H, **Zhou JJ**. Exact variance component tests for longitudinal microbiome studies. Genet Epidemiol. 2019 Apr;43(3):250-262. PMCID: PMC6416054.
    c. **Zhou JJ**, Hu T, Qiao D, Cho MH, Zhou H. Boosting gene mapping power and efficiency with efficient exact variance component tests of single nucleotide polymorphism sets. Genetics. 2016 Nov;204(3):921-931. PMCID: PMC5105869.
    d. Doubleday K, Zhou H, Fu H, **Zhou JJ**. An Algorithm for Generating Individualized Treatment Decision Trees and Random Forests. J Comput Graph Stat. 2018;27(4):849-860. 2018 Jun 14. PMCID: PMC7286561.

2. Identification of risk factors underly the development of diabetes complications. I have devoted myself to studying clinical and biological aspects of diabetes and its complications in the past five years. Taking advantage of my quantitative background, my group has conducted several secondary analyses of clinical trials. The findings highlighted the importance of intraindividual glycemic variability as an independent risk factor to diabetes complications. Together, they allow me to move closer to my career goal: at the interface of statistics, genetics, and biomedical informatics and better utilize “big” health-related data for personalized diabetes care.
    a. **Zhou JJ**, Koska J, Bahn G, Reaven P. Fasting Glucose Variation Predicts Microvascular Risk in ACCORD and VADT. J Clin Endocrinol Metab. 2021 Mar 25;106(4):1150-1162. PMCID: PMC7993576.
    b. **Zhou JJ,** Schwenke DC, Bahn G, Reaven P; VADT Investigators. Glycemic Variation and Cardiovascular Risk in the Veterans Affairs Diabetes Trial. Diabetes Care. 2018 Oct;41(10):2187-2194. 2018 Aug 6. PMCID: PMC6150432.
    c. **Zhou JJ**, Koska J, Bahn G, Reaven P. Glycaemic variation is a predictor of all-cause mortality in the Veteran Affairs Diabetes Trial. Diab Vasc Dis Res. 2019 Mar;16(2):178-185. PMCID: PMC7380497.

3. Identification of genetic determinants of complex diseases. Besides developing quantitative tools, I have made substantial efforts to understand genetic factors underlying complex diseases utilizing advanced computational tools and methodologies. We are the first to estimate heritability of COPD and related phenotypes using a GWAS study of 10,000 individuals.
    a. **Zhou JJ**, Cho MH, Castaldi PJ, Hersh CP, Silverman EK, Laird NM. Heritability of chronic obstructive pulmonary disease and related phenotypes in smokers. Am J Respir Crit Care Med. 2013 Oct 15;188(8):941-7. PMCID: PMC3826281.

b. Klimentidis YC, Arora A, Newell M, **Zhou JJ**, Ordovas JM, Renquist BJ, Wood AC. Phenotypic and Genetic Characterization of Lower LDL Cholesterol and Increased Type 2 Diabetes Risk in the UK Biobank. Diabetes. 2020 Oct;69(10):2194-2205 PMCID: PMC7506834.
c. **Zhou JJ**, Zhai J, Zhou H, Chen Y, Guerra S, Robey I, Weinstock GM, Weinstock E, Dong Q, Knox KS, Twigg HL 3rd. Supraglottic Lung Microbiome Taxa Are Associated with Pulmonary Abnormalities in an HIV Longitudinal Cohort. Am J Respir Crit Care Med. 2020 Dec 15;202(12):1727-1731. PMCID: PMC7737582.

4. Applied Mathematics. Combinatorics and graph theory are new fields in modern mathematics. Its recent spectacular growth is motivated by computer science, for instance, for the construction and analysis of computational algorithms. In my earlier papers, I studied enumeration, one of the most basic and important aspects of combinatorics. Some of the counting procedures in enumerative combinatorics are widely used in phylogenetic studies. Genocchi numbers are widely used for enumeration. We are the first to introduce a q-analog of Genocchi numbers through a q-analog of Seidels triangle and showed that these q-Genocchi numbers have interesting combinatorial interpretations such as alternating pistols, alternating permutations, and skew Young tableaux.
    a. Yan G, Yang L, **Zhou JJ**. The Zrank conjecture and restricted Cauchy matrix. Linear Algebra and its Applications. 2005; 411:371-385.
    b. Zeng J, **Zhou JJ**. Applications of Waring's formula to some identities of Chebyshev polynomials. Fibonacci Quarterly. 2006; 44.2:117-120.
    c. Zeng J, **Zhou JJ**. A q-analog of the Seidel generation of Genocchi numbers. European Journal of Combinatorics. 2006; 27:364-381.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47795663/?sort=date&direction=ascending

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Allan Mackenzie-Graham, Ph.D.**

eRA COMMONS USER NAME (credential, e.g., agency login): MACKENZIEG2

POSITION TITLE: **Associate Professor, Neurology**

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
University of California, Los Angeles, CA	B.S.	12/1996	Neuroscience
University of California, Los Angeles, CA	Ph.D.	04/2006	Neuroscience
University of California, Los Angeles, CA	Post-doc	06/2008	Neurology
University of California, Los Angeles, CA	Post-doc	06/2010	Neurology

A. Personal Statement

My primary interests are in the mechanisms underlying neurodegeneration and how to ameliorate it therapeutically. I have a broad background in neuroscience, imaging, and histology and I apply all of these skills to the end of understanding neurodegeneration in the normal aging process and neurological disease, specifically multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), the most commonly used animal model of MS. My research involves the analysis of mouse brain magnetic resonance imaging (MRI) using voxel-based morphometry (VBM) and Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging-compatible Tissue-hYdrogel (CLARITY) imaging using atlasing techniques to evaluate the structural changes that occur in the brain as the result of disease and treatment. We have recently published this work in the Multiple Sclerosis Journal (Meyer 2109). In parallel, my lab studies localized gray matter changes using VBM in patients with MS. We strive to understand the relationship between gray matter atrophy and specific clinical disabilities in order to identify treatments to stop gray matter loss and prevent disability. This work was published in JAMA Neurology (MacKenzie-Graham 2016), Brain & Behavior (MacKenzie-Graham 2018), and Annals of Clinical and Translational Neurology (Voskuhl 2022).

I am very excited about working with Dr. Voskuhl on this groundbreaking proposal, Neurodegeneration Underlying Distinct Disabilities in Multiple Sclerosis Using a Cell-Specific, Region-Specific, and Sex-Specific Approach. The proposal leverages my lab's strengths in MRI acquisition, processing, and analysis, as well as our extensive collaborative history with Dr. Voskuhl, comprising many papers and grants over the years.

Citations I would like to highlight

1. Meyer CE, Gao JL, Cheng JY, Oberoi MR, Johnsonbaugh H, Lepore S, Kurth F, Thurston MJ, Itoh N, Patel KR, Voskuhl RR, **MacKenzie-Graham A**. (2019) Axonal Damage in Spinal Cord Is Associated with Gray Matter Atrophy in Sensorimotor Cortex in Experimental Autoimmune Encephalomyelitis. Mult Scler. 2019 Mar 7:1352458519830614. doi: 10.1177/1352458519830614. PMID: 30843756
2. Meyer CE, Kurth F, Lepore S, Gao JL, Johnsonbaugh H, Oberoi MR, Sawiak SJ, **MacKenzie-Graham A**. (2017) In Vivo Magnetic Resonance Images Reveal Neuroanatomical Sex Differences Through the Application of Voxel-Based Morphometry in C57BL/6 Mice. NeuroImage 163:197-205. PMCID: PMC5716897
3. Voskuhl RR, Patel K, Paul F, Gold SM, Scheel M, Kuchling J, Cooper G, Asseyer S, Chien C, Brandt AU, Meyer CE, **MacKenzie-Graham A.** (2020) Sex Differences in Brain Atrophy in Multiple Sclerosis. Biol Sex Differ. 2020 Aug 28;11(1):49. doi: 10.1186/s13293-020-00326-3. PMID: 32859258 PMCID: PMC7456053
4. **MacKenzie-Graham A**, Kurth F, Itoh Y, Wang HJ, Montag MJ, Elashoff R, Voskuhl RR. (2016) Disability-Specific Atlases of Gray Matter Loss in Relapsing-Remitting Multiple Sclerosis. JAMA Neurology 73(8):944-53. PMID: 27294295, PMCID: PMC6415681

Ongoing and completed projects I would like to highlight

NIH R01 NS121761 (MacKenzie-Graham/Shattuck MPI) 04/01/2022-03/31/2027

A Toolkit for Analysis and Visualization of Preclinical Rodent Neuroimaging Experiments

Goal: The goal of this project is to develop an open-source suite of software for the processing, analysis, and

visualization of rodent brain imaging data, which will enhance the ability of neuroscience researchers to perform preclinical studies on rodent models. This software will provide advanced capabilities for analyzing multimodal imaging data, including imagery from magnetic resonance imaging and optical microscopy studies, with the goal of improving understanding of processes and mechanisms underlying neurological diseases and disorders and their potential treatment.
Role: Multiple Principal Investigator

NIH R21NS121806 (MacKenzie-Graham PI) 04/01/21-09/30/2022
The Mechanism of Gray Matter Atrophy in Experimental Autoimmune Encephalomyelitis
Goal: To determine the role of oxidative stress in gray matter atrophy in EAE.
Role: Principal Investigator

NIH R01HD100298 (MacKenzie-Graham/Arnold/Lerch MPIs) 04/01/2020-03/31/2025
Coupling Neuroimaging with CLARITY and Single-Cell Genomics to Dissect Sex Differences in the Developing Brain
Goal: This project establishes sex-biasing effects of sex hormones and sex chromosomes on localized brain regions across the entire brain at specific times of life and investigates cellular and molecular mechanisms operating in a sex-specific manner at salient sites of sexual differentiation.
Role: Multiple Principal Investigator

Conrad N. Hilton Foundation #18394 (Voskuhl PI) 07/01/19-06/30/22
Disability-Specific Disease Modifying Treatments (DMTs) in Multiple Sclerosis (MS)
Goal: Investigation of disability-specific treatments in EAE, MS, and brain aging.
Role: Co-Investigator

NIH R01NS086981 (MacKenzie-Graham PI) 03/01/15-02/28/20
Bringing CLARITY to EAE
Goal: To determine the neuropathologies underlying gray matter atrophy in EAE.
Role: Principal Investigator

National Multiple Sclerosis Society #PP-1805-31001 (Gold PI) 11/1/18-10/31/19
UCLA Subcontract with Charité – Universitätsmedizin Berlin
Sex Differences in Neurodegeneration in CIS and Early Stage MS
Goal: Assess sex-specific disability progression and brain atrophy using an existing MS dataset in Berlin.
Role: Co-Investigator (UCLA Subcontract with Charité – Universitätsmedizin Berlin)

B. Positions, Scientific Appointments, and Honors

Employment

2017-present	Associate Professor, Brain Mapping Center, Neurology, UCLA, Los Angeles, CA
2015-present	Joint appointment in Physics and Biology in Medicine IDP, UCLA, Los Angeles, CA
2015-present	Joint appointment in Neuroscience IDP, UCLA, Los Angeles, CA
2010-2017	Assistant Professor, Brain Mapping Center, Neurology, UCLA, Los Angeles, CA
2008-2010	Postdoctoral Scholar (Jeffry Alger mentor), Neurology, UCLA, Los Angeles, CA
2006-2008	Postdoctoral Scholar (Arthur Toga mentor), Neurology, UCLA, Los Angeles, CA
1998-2006	Graduate Student (Arthur Toga mentor), Neuroscience, UCLA, Los Angeles, CA
1995-1998	Staff Research Associate, Neurology, UCLA, Los Angeles, CA

Other Experience and Professional Memberships

2019	ad hoc reviewer, NIH Peer Review Committee: CHHD-R
2019	ad hoc reviewer, NIH Peer Review Committee: CNBT
2018	ad hoc reviewer, NIH Peer Review Committee: MEDI
2018-present	Organization for the Study of Sex Differences, Member
2016-present	International Society for Neurochemistry, Member
2015-present	American Society for Neurochemistry, Member
2011-present	International Society for Magnetic Resonance in Medicine, Member
2012-2016	Laboratory of Neuroendocrinology, Minority Training Committee, Chair
2010-present	Laboratory of Neuroendocrinology, Member
1999-present	Society for Neuroscience, Member

Honors

2009 Mazziotta Award: Postdoctoral Scholar
2008-2011 NMSS Postdoctoral Fellow, National Multiple Sclerosis Society
1999-2003 METPAC Fellow, Minority Conference Fellowship Program, Society for Neuroscience

C. Contribution to Science

1. My laboratory studies the structural changes that occur in the central nervous system as the result of aging and disease, specifically, the relationship between GM atrophy and the neuropathologies that underlie it. We use cross-modality correlation to uncover these associations. The studies below document a strong correlation between GM atrophy measured by MRI and neuronal loss as measured by histology in EAE. Our early findings demonstrated that gray matter atrophy in the cerebellum and Purkinje cell loss were strongly associated in mice with EAE. More recently, we used CLARITY to observe a very strong correlation between not only neuronal loss and gray matter atrophy in the cerebral cortex, but also between axonal transection in the spinal cord and gray matter atrophy in the cerebral cortex. This work was the first use of the CLARITY technique in an animal model of disease.

    a. Meyer CE, Gao JL, Cheng JY, Oberoi MR, Johnsonbaugh H, Lepore S, Kurth F, Thurston MJ, Itoh N, Patel KR, Voskuhl RR, **MacKenzie-Graham A**. (2019) Axonal Damage in Spinal Cord Is Associated with Gray Matter Atrophy in Sensorimotor Cortex in Experimental Autoimmune Encephalomyelitis. Mult Scler. 2019 Mar 7:1352458519830614. doi: 10.1177/1352458519830614. PMID: 30843756
    b. Spence RD, Kurth F, Itoh N, Mongerson CRL, Wailes SH, Peng MS, **MacKenzie-Graham AJ**. (2014) Bringing CLARITY to Gray Matter Atrophy. NeuroImage 101:625-32 PMCID: PMC4437539
    c. **MacKenzie-Graham A**, Tiwari-Woodruff SK, Sharma G, Aguilar C, Vo KT, Strickland LV, Morales L, Fubara B, Martin M, Jacobs RE, Johnson GA, Toga AW, Voskuhl RR (2009) Purkinje Cell Loss in Experimental Autoimmune Encephalomyelitis. NeuroImage, 48(4):637-651. PMCID: PMC2754586
    d. **MacKenzie-Graham A,** Tinsley MR, Shah KP, Aguilar C, Strickland LV, Boline J, Martin M, Morales L, Shattuck DW, Jacobs RE, Voskuhl RR, Toga AW. (2006) Cerebellar Cortical Atrophy in Experimental Autoimmune Encephalomyelitis. Neuroimage 32(3):1016-23. PMID: 16806982

2. We are particularly interested in how to prevent, ameliorate, and/or repair damage to the CNS. In collaboration with Dr. Rhonda Voskuhl from the UCLA MS Program and with the intellectual support of the Laboratory of Neuroendocrinology (LNE), we have studied the effects of sex hormones, namely estriol and testosterone, on gray matter atrophy in EAE and MS, respectively. Both estriol and testosterone are promising treatments for MS. In a testosterone-treatment trial in men with MS, we found not only a decrease in gray matter atrophy after just six months, but also a localized increase in gray matter concentration after 12 months of treatment. We have also observed gray matter preservation by MRI and decreased myelin disruption by diffusion tensor imaging (DTI) in EAE mice treated with estrogen. Recently, we studied the association between voxelwise gray matter atrophy and clinical disability, discovering that gray matter loss in clinically eloquent regions are correlated with the paced auditory serial addition test (PASAT), 9-hole peg test (9-HPT), and the bowel and bladder subscale of the Kurtzke expanded disability status scale (EDSS).

    a. Voskuhl R, Kuhle J, Siddarth P, Itoh N, Patel K, **MacKenzie-Graham A**. (2022) Decreased Neurofilament Light Chain Levels In Estriol-Treated Multiple Sclerosis. Ann Clin Transl Neurol. 2022 Jun 29. doi:10.1002/acn3.51622. Online ahead of print. PMID: 35770318
    b. **MacKenzie-Graham A**, Brook J, Kurth F, Itoh Y, Meyer C, Montag MJ, Wang HJ, Elashoff R, Voskuhl RR. (2018) Estriol-Mediated Neuroprotection in Multiple Sclerosis Localized by Voxel-Based Morphometry. Brain Behav. 2018 Aug 24:e01086. doi: 10.1002/brb3.1086. PMID: 30144306, PMCID: PMC6160650
    c. **MacKenzie-Graham A**, Kurth F, Itoh Y, Wang HJ, Montag MJ, Elashoff R, Voskuhl RR. (2016) Disability-Specific Atlases of Gray Matter Loss in Relapsing-Remitting Multiple Sclerosis. JAMA Neurology 73(8):944-53. PMID: 27294295, PMCID: PMC6415681

d. Voskuhl RR, Wang H, Wu TC, Sicotte NL, Nakamura K, Kurth F, Itoh N, Bardens J, Bernard JT, Corboy JR, Cross AH, Dhib-Jalbut S, Ford CC, Frohman EM, Giesser B, Jacobs D, Kasper LH, Lynch S, Parry G, Racke MK, Reder AT, Rose J, Wingerchuk DM, **MacKenzie-Graham AJ**, Arnold DL, Tseng CH, Elashoff R. (2015) Estriol Combined with Glatiramer Acetate for Women with Relapsing-Remitting MS: A Randomised, Placebo-Controlled, Phase 2 Trial. Lancet Neurology Jan;15(1):35-46. PMID: 26621682

3. My laboratory is also interested in understanding sex differences in the brain, particularly the contribution of sex chromosomes to structural differences, as our recent grant funding demonstrates (NIH R01HD100298 and NIH R21HD090637). Early in my career I developed a framework for evaluating differences in structural anatomy in the mouse. More recently, we have used VBM to visualize changes in both the mouse and human brains. For VBM in the mouse we developed species- and in vivo-specific tissue probability maps (TPMs) to provide more accurate tissue segmentation. Our results demonstrated distinct neuroanatomical regions that were sexually dimorphic in the adult C57BL/6 mouse brain. Similarly, we found sexual dimorphism in the pattern of GM loss in the brain during MS by comparing female MS patients with female healthy controls and male MS patients with male healthy controls.

   a. Voskuhl RR, Patel K, Paul F, Gold SM, Scheel M, Kuchling J, Cooper G, Asseyer S, Chien C, Brandt AU, Meyer CE, **MacKenzie-Graham A.** (2020) Sex Differences in Brain Atrophy in Multiple Sclerosis. Biol Sex Differ. 2020 Aug 28;11(1):49. doi: 10.1186/s13293-020-00326-3. PMID: 32859258 PMCID: PMC7456053
   b. Meyer CE, Kurth F, Lepore S, Gao JL, Johnsonbaugh H, Oberoi MR, Sawiak SJ, **MacKenzie-Graham A**. (2017) In Vivo Magnetic Resonance Images Reveal Neuroanatomical Sex Differences Through the Application of Voxel-Based Morphometry in C57BL/6 Mice. NeuroImage 163:197-205. PMCID: PMC5716897
   c. Kurth F, **Mackenzie-Graham A**, Toga AW, Luders E. (2014) Shifting Brain Asymmetry: The Link Between Meditation and Structural Lateralization. Social Cognitive and Affective Neuroscience 10(1):55-61 PMID: 24643652
   d. **MacKenzie-Graham A**, Lee EF, Dinov I, Bota M, Shattuck DW, Ruffins S, Yuan H, Konstantinidis F, Pitiot A, Ding Y, Hu G, Jacobs RE, Toga AW (2004) A Multimodal, Multidimensional Atlas of the C57BL/6 Mouse Brain. Journal of Anatomy 204:93-102. PMCID: PMC1571243

4. As part of my doctoral training, I learned how to use sophisticated MRI analysis techniques to study natural variability in mice. In my first postdoctoral fellowship, I learned how to measure the effect of disease on brain morphology using MRI and in my second fellowship I learned how to collect MR images and correlate them with neuropathological changes. I successfully managed the Mouse Atlas Project (MAP) and served as the primary liaison and scientific advisor for the Laboratory of Neuro Imaging (LONI) to the Mouse Biomedical Informatics Research Network (MBIRN). A significant aspect of this work was the development of a mechanism for documenting data provenance for the documentation and validation of multiple kinds of imaging data.

   a. **MacKenzie-Graham A**, Payan A, Dinov I, Van Horn JD, Toga AW. (2008) Neuroimaging Data Provenance Using the LONI Pipeline Workflow Environment. In: Lecture Notes in Computer Science: International Provenance and Annotation Workshop 2008, Freire J, Koop D, and Moreau L (Eds). Springer-Verlag, pp 4145:208-220. LNCS 4145: 148–161.
   b. **MacKenzie-Graham A**, Van Horn JD, Payan A, Neu SC, Crawford KL, Toga AW (2008) Provenance in Neuroimaging. NeuroImage, 42(1):178-95. PMCID: 2664747
   c. **MacKenzie-Graham A**, Lee EF, Dinov ID, Yuan H, Jacobs RE, Toga AW. (2007) Multimodal, Multidimensional Models of Mouse Brain. Epilepsia, 48(Suppl. 4):75-81. PMCID: PMC3192853
   d. **MacKenzie-Graham A**, Jones ES, Shattuck DW, Dinov I, Bota M, Toga AW. (2003) The Informatics of a C57BL/6J Mouse Brain Atlas. Neuroinformatics 1:397-410. PMID: 15043223

Complete List of Published Work in MyBibliography
http://www.ncbi.nlm.nih.gov/sites/myncbi/allan.mackenzie-graham.1/bibliography/40421703/public/?sort=date&direction=ascending

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Prabha Siddarth

eRA COMMONS USER NAME (credential, e.g., agency login): siddarth2

POSITION TITLE: Research Statistician

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Madras, Madras, India	B.Sc.	06/1982	Chemistry
Indian Institute of Technology, Madras, India	M.Sc.	06/1984	Chemistry
Indian Institute of Technology, Madras, India	Ph.D.	06/1988	Chemistry
University of California, Los Angeles	M.S.	06/1999	Biostatistics

A. Personal Statement

I am a Research Statistician in the Department of Psychiatry and Biobehavioral Sciences at the University of California, Los Angeles (UCLA). As a biostatistician, I have extensive experience in experimental design, statistical analysis, and integration of complex multimodal data from translational, genomic, imaging and observational longitudinal studies as well as behavioral intervention trials, specifically in the area of neurological disorders. I have contributed significantly to research in behavioral, psychiatric and physiological aspects of depression, aging, cognitive functioning and Alzheimer's disease using multimodal imaging, and my work, which has appeared in top-tier journals in psychiatry (e.g. PNAS, Archives of General Psychiatry, Brain, the American Journal of Psychiatry), is highly cited, giving me an h-index of 53 and an i-10-index of 128. I have also served as the statistician in a multi-site study of clinical, cognitive, language, neuropsychological and psychiatric evaluation of patients with non-epileptic seizures recruited from 5 tertiary medical centers. I have a longstanding productive collaborative relationship with many Principal Investigators both within UCLA and outside of UCLA and my involvement has included statistical consultation regarding design, data collection as well as data analysis for various papers, presentations at scientific conferences, and participation in writing manuscripts. Further, I have also collaborated with many other investigators in clinical trials and other longitudinal studies and have recently published a paper with the PI of the current proposal. In the current project, I will participate in the analysis, interpretation, and publication of findings. Some recent publications where my contributions have been critical to the success of the projects are highlighted below:

a. Voskuhl R, Kuhle J, **Siddarth P**, Itoh N, Patel K, MacKenzie-Graham A. Decreased neurofilament light chain levels in estriol-treated multiple sclerosis. Ann Clin Transl Neurol. 2022 Jun 29. doi: 10.1002/acn3.51622. Epub ahead of print. PMID: 35770318.
b. Lavretsky H, Laird KT, Krause-Sorio B, Heimberg BF, Yeargin J, Grzenda A, Wu P, Thana-Udom K, Ercoli LM, **Siddarth P**. A Randomized Double-Blind Placebo-Controlled Trial of Combined Escitalopram and Memantine for Older Adults With Major Depression and Subjective Memory Complaints. Am J Geriatr Psychiatry. 2020 Feb;28(2):178-190. PMCID: PMC6997044
c. **Siddarth P**, Li Z, Miller KJ, Ercoli LM, Merrill DA, Henning SM, Heber D, Small GW. Randomized placebo-controlled study of the memory effects of pomegranate juice in middle-aged and older adults. Am J Clin Nutr. 2020;111(1):170–177 .
d. **Siddarth P.** Sparse data and use of logistic regression. Epilepsia (2018) 59, 1085.

B. Positions, Scientific Appointments, and Honors

Employment

2008-present	Research Statistician	University of California, Los Angeles
2003-2008	Associate Research Statistician	University of California, Los Angeles
1999-2003	Assistant Research Statistician	University of California, Los Angeles
1997-1999	Statistician	University of California, Los Angeles
1994-1997	Adjunct Professor	University of British Columbia
1991-1993	Senior Postdoctoral Fellow	California Institute of Technology
1988-1991	Postdoctoral Fellow	California Institute of Technology

Honors

2020-Present	Editorial Board, Journal of Geriatric Psychiatry and Neurology
2013-Present	Associate Editor, Epilepsia
2010-Present	Statistics Editor, Journal of Pediatric Epilepsy
2007	Young Investigators' Award, International Epilepsy Congress, Singapore
1999	Fellow, Delta Omega Society
1998	Raymond D. Goodman Scholarship for academic excellence, UCLA
1991	NATO Advanced Study Institute Fellowship, Caltech
1986 – 1988	CSIR Senior Research Fellowship, IIT
1984 1986	Junior Research Fellowship, IIT

C. Contributions to Science

I am extremely active in collaborative work, and am involved in projects in diverse areas but my primary field of application is psychiatry. The following is a brief description of my most significant contributions to science.

1. Research Relating to Memory, Aging and Late-life Depression
   I am the principal statistician for the UCLA Longevity Center and the Late-Life Depression, Stress and Wellness Program. Research in these programs have focused on identifying neuroanatomical correlates of depression, Alzheimer's disease, mild cognitive impairment to elucidate disease mechanisms, and on identifying cerebral abnormalities indicating disease-related predisposition in unaffected individuals biologically at-risk. Early detection has focused on imaging techniques; in addition to the imaging studies, I have been active in designing research studies to investigate the efficacy of mind-body intervention interventions to ameliorate depressive symptoms, and arrest cognitive decline in older adults. In all these studies, I play a fundamental role in study design, grant writing, data analyses, manuscript preparation and discussion of future studies, and am the liaison to collaborative laboratories.

   a. **Siddarth P**, Funes CM, Laird KT, Ercoli L, Lavretsky H. Predictors of Cognitive Improvement Following Treatment for Late-Life Depression. J Geriatr Psychiatry Neurol. 2020 Mar 25:891988720915515.
   b. Laird KT, Lavretsky H, St Cyr N, **Siddarth P.** Resilience predicts remission in antidepressant treatment of geriatric depression. Int J Geriatr Psychiatry. 2018 Dec;33(12):1596-1603 PMCID: PMC6246780
   c. **Siddarth P**, Rahi B, Emerson ND, Burggren AC, Miller KJ, Bookheimer S, Lavretsky H, Dobkin B, Small G, Merrill DA. Physical Activity and Hippocampal Sub-Region Structure in Older Adults with Memory Complaints. J Alzheimers Dis. 2018 61, 1089-1096 PMCID: PMC6461048
   d. **Siddarth P**, Thana-udom K, Ojha R, Merrill DA, Dzierzewski J, Miller K, Small GW and Ercoli L. Sleep quality, neurocognitive performance, and memory self-appraisal in middle-aged and older adults with memory complaints. Int Psychogeriatr. 2020. PMID: 32985406 PMCID: PMC8004546

2. Research Relating to Pediatric Epilepsy and Schizophrenia
   In collaboration with Dr. Caplan, I have been able to demonstrate neuropsychiatric comorbidities and imaging abnormalities in children with epilepsy with average IQ scores, even in epilepsy syndromes previously thought to be "benign" and in childhood schizophrenia. This research has involved various complex imaging modalities, including deformation-based morphometry, diffusion tensor imaging and

proton magnetic resonance spectroscopy. As an example, using deformation-based morphometry (a method for identifying macroscopic anatomical differences among the brains of different groups of subjects by first spatially normalizing the structural magnetic resonance images of a number of subjects so that they all conform to the same stereotaxic space and then applying multivariate statistics to the ensuing parameters describing the estimated nonlinear deformations), we showed that children with absence epilepsy did not demonstrate the normal regional age-related changes involving a decrease in cortical thickness and increase in sulcal depth and that they used different brain regions to perform cognitive functions compared to healthy controls. Similarly, children with complex partial seizures also exhibited abnormal age related patterns of cortical thickness and sulcal depth in a diversity of regions. These findings have far-reaching implications for both the children with epilepsy and their health care providers. In all these publications, I played a fundamental role in the conceptualization, data analysis and manuscript preparation.

a. Tosun D, **Siddarth P**, Levitt J, Caplan R. Cortical Thickness and Sulcal Depth: Insights on Development and Psychopathology in Pediatric Epilepsy. BJPsych Open 2015 1, 129-35. PMCID: PMC4995587
b. Doss J, Caplan R, **Siddarth P**, Bursch B, Falcone T, Forgey M, Hinman K, Curt LaFrance W Jr, Laptook R, Shaw R, Weisbrot D, Willis M, Plioplys S. Risk factors for learning problems in youth with psychogenic non-epileptic seizures. 2017 Epilepsy Behav. 70(Pt A):135-139.
c. Jones JE, **Siddarth P**, Almane D, Gurbani S, Hermann BP, Caplan R. Identification of risk for severe psychiatric comorbidity in pediatric epilepsy. Epilepsia 2016 57, 1817-1825. PMCID: PMC5868343
d. Lin JJ, **Siddarth P**, Riley JD, Gurbani SG, Ly R, Yee VW, Levitt JG, Toga AW, Caplan R. Neurobehavioral comorbidities of pediatric epilepsies and thalamic structural abnormalities. Epilepsia. 2013 **54**, 2116. PMCID: PMC4259153.

3. Other Research Projects
We have recently started a collaborative effort with the Gallup organization to investigate risk factors for memory problems and depressive symptoms. I have spearheaded the data analyses and manuscript preparation for these projects. These projects comprise data from tens of thousands of subjects (N is approximately 40,000) and the analyses have consequently been challenging and have required development of sophisticated data analytical techniques. I am also the principal statistician for a recently concluded multi-site project to investigate neural and psychosocial mechanisms as well as interventions for non-epileptic seizures in children and adolescents. This multi-site study involves collaborators from Children's Memorial Hospital, Chicago, Children's National Medical center, Washington, D.C., University of Pittsburgh Medical Center, University of Indiana, Stanford University and UCLA. I also spearheaded a recent project with UCLA Recreation, collecting and analyzing data provided by 281 volunteer participants in an employee wellness program, demonstrating a clear link between improvement in physical health and improvements in mental health, quality of life, stress, and energy.

a. Small GW, **Siddarth P**, Ercoli LM, Chen ST, Merrill DA, Torres-Gil F Healthy behavior and memory reports in young, middle-aged, and older adults. Int Psychogeriatr. (2013) **25**, 981.
b. Chen ST, **Siddarth P**, Ercoli LM, Merrill DA, Torres-Gil F, Small GW Modifiable Risk Factors for Alzheimer's Disease and Subjective Memory Impairment Across Age Groups. PLoS One. (2014) 9(6):e98630. PMCID: PMC4045888.
c. Plioplys S, Doss J, **Siddarth P**, Bursch B, Falcone T, Forgey M, Hinman K, LaFrance WC Jr, Laptook R, Shaw RJ, Weisbrot DM, Willis MD, Caplan R. Risk factors for comorbid psychopathology in youth with psychogenic nonepileptic seizures. Seizure (2016) 38, 32-7.
d. Emerson ND, Merrill DA, Shedd K, Bilder RM, **Siddarth P**. Effects of an employee exercise programme on mental health. Occup Med (Lond). 2016 Aug 23 kqw120. doi: 10.1093/occmed/kqw120

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/prabha.siddarth.2/bibliography/public

Name: Voskuhl, Rhonda Renee
E-Commons ID: VOSKUHL2

OTHER SUPPORT – Project/Proposal

1. ACTIVE

Project/Proposal Title	**Non-invasive Imaging of Brain Infiltrating T Lymphocytes in a Mouse Model of Experimental Autoimmune Encephalomyelitis with PET**
Major Goals	To image inflammation in vivo in EAE
Status of Support	Active
Project Number	R01NS112287
Name of PD/PI	Clark, Peter M
Source of Support	NIH-NINDS National Institute of Neurological Disorders and Stroke
Primary Place of Performance	University of California, Los Angeles
Project/Proposal Support Start Date	5/15/2019
Project/Proposal Support End Date	2/29/2024
Annual Direct Costs	$218,750
Person Months Per Budget Period	**Year** / **Cal** / **Acad** / **Sum** **2023** 0.60 **2024** 0.60

Project/Proposal Title	**Novel Estrogen Receptor Beta Ligand Treatment for Neuroprotection**
Major Goals	Preclinical studies of a novel ER beta ligand for development as a treatment for MS
Status of Support	Active
Project Number	20205124
Name of PD/PI	Voskuhl, Rhonda Renee
Source of Support	Yuyu Pharma, Inc. (Korea, Republic of)
Primary Place of Performance	University of California, Los Angeles
Project/Proposal Support Start Date	1/16/2021
Project/Proposal Support End Date	7/16/2023
Annual Direct Costs	$48,800
Person Months Per Budget Period	**Year** / **Cal** / **Acad** / **Sum** **2023** 1.20

Name: Voskuhl, Rhonda Renee
E-Commons ID: VOSKUHL2

Project/Proposal Title	**Neuroprotection in MS: A Cell-Specific and Region-Specific Transcriptomics Approach**
Major Goals	Understanding neurodengerative mechanisms in preclinical model of MS
Status of Support	Active
Project Number	R01NS109670
Name of PD/PI	Voskuhl, Rhonda Renee
Source of Support	NIH-NINDS National Institute of Neurological Disorders and Stroke
Primary Place of Performance	University of California, Los Angeles
Project/Proposal Support Start Date	9/30/2018
Project/Proposal Support End Date	5/31/2023
Annual Direct Costs	$284,984
Person Months Per Budget Period	**Year** / **Cal** / **Acad** / **Sum** **2023** / 3.60 / /

Project/Proposal Title	**The Mechanism of Gray Matter Atrophy in Experimental Autoimmune Encephalomyelitis**
Major Goals	To determine the role of oxidative stress in gray matter atrophy in EAE.
Status of Support	Active
Project Number	R21NS121806
Name of PD/PI	Mackenzie-Graham, Allan
Source of Support	NIH-NINDS National Institute of Neurological Disorders and Stroke
Primary Place of Performance	University of California, Los Angeles
Project/Proposal Support Start Date	4/1/2021
Project/Proposal Support End Date	9/30/2022
Annual Direct Costs	$125,000
Person Months Per Budget Period	**Year** / **Cal** / **Acad** / **Sum** **2022** / 0.60 / /

2. PENDING

None

3. IN-KIND CONTRIBUTIONS / FOREIGN SUPPORTS

None

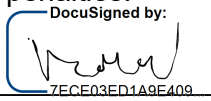
Name: Voskuhl, Rhonda Renee
E-Commons ID: VOSKUHL2

4. OVERLAP

None

5. PI Certification

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

DocuSigned by:

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Signature

June 28, 2022

Date

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Rhonda	R.	Voskuhl	MD	PD/PI	203,700.00	6.6			112,035.00	43,133.00	155,168.00
2 .	Dr.	Yuichiro		Itoh	PhD	Co-Investigator	107,900.00	4.2			37,765.00	18,240.00	56,005.00
3 .	Dr.	Jin		Zhou	PhD	Co-Investigator	160,000.00	0.6			8,000.00	3,080.00	11,080.00
4 .	Dr.	Allan		MacKenzie-Graham	PhD	Co-Investigator	137,900.00	1.8			20,685.00	7,964.00	28,649.00
5 .	Dr.	Prabha		Siddarth	PhD	Co-Investigator	153,697.00	0.6			7,685.00	3,712.00	11,397.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **262,299.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6			36,971.00	1,479.00	38,450.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Lab Technician	12			74,500.00	40,081.00	114,581.00
1	MRI Lab Technician	3.6			15,485.00	8,331.00	23,816.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**176,847.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**439,146.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	24,426.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animals	45,000.00
9. Sequencing Core facility	25,000.00
10. Mitochondrial function / Synaptosome Core	8,000.00
11. MRI in mice	25,000.00
12. DOMSTAT	3,000.00
13. Tuition Fees	17,857.00
14. Technology Infrastructure Fees (TIF)	1,556.00
Total Other Direct Costs	**149,839.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**591,985.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research On Campus	56	574,128.00	321,512.00
		Total Indirect Costs	**321,512.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Janet Turner, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**913,497.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	913,497.00

L. Budget Justification*	File Name:
	Budget_Justification_RV1071243866.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Rhonda	R.	Voskuhl	MD	PD/PI	203,700.00	6.6			112,035.00	43,133.00	155,168.00
2 .	Dr.	Yuichiro		Itoh	PhD	Co-Investigator	107,900.00	4.2			37,765.00	18,240.00	56,005.00
3 .	Dr.	Jin		Zhou	PhD	Co-Investigator	160,000.00	0.6			8,000.00	3,080.00	11,080.00
4 .	Dr.	Allan		MacKenzie-Graham	PhD	Co-Investigator	137,900.00	1.8			20,685.00	7,964.00	28,649.00
5 .	Dr.	Prabha		Siddarth	PhD	Co-Investigator	153,697.00	0.6			7,685.00	3,712.00	11,397.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **262,299.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6			36,971.00	1,479.00	38,450.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Lab Technician	12			74,500.00	40,081.00	114,581.00
1	MRI Lab Technician	3.6			15,485.00	8,331.00	23,816.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**176,847.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**439,146.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT13677549
Funding Opportunity Number: RFA-NS-22-038 . Received Date: 2022-07-08T17:54:10.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	24,426.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animals	45,000.00
9. Sequencing Core facility	25,000.00
10. Mitochondrial function / Synaptosome Core	8,000.00
11. MRI in mice	25,000.00
12. DOMSTAT	3,000.00
13. Tuition Fees	17,857.00
14. Technology Infrastructure Fees (TIF)	1,556.00
Total Other Direct Costs	**149,839.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**591,985.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research On Campus	56	574,128.00	321,512.00
		Total Indirect Costs	**321,512.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Janet Turner, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**913,497.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	913,497.00

L. Budget Justification*	File Name:
	Budget_Justification_RV1071243866.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Rhonda	R.	Voskuhl	MD	PD/PI	203,700.00	6.6			112,035.00	43,133.00	155,168.00
2 .	Dr.	Yuichiro		Itoh	PhD	Co-Investigator	107,900.00	4.2			37,765.00	18,240.00	56,005.00
3 .	Dr.	Jin		Zhou	PhD	Co-Investigator	160,000.00	0.6			8,000.00	3,080.00	11,080.00
4 .	Dr.	Allan		MacKenzie-Graham	PhD	Co-Investigator	137,900.00	1.8			20,685.00	7,964.00	28,649.00
5 .	Dr.	Prabha		Siddarth	PhD	Co-Investigator	153,697.00	0.6			7,685.00	3,712.00	11,397.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **262,299.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6			36,971.00	1,479.00	38,450.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Lab Technician	12			74,500.00	40,081.00	114,581.00
1	MRI Lab Technician	3.6			15,485.00	8,331.00	23,816.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**176,847.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**439,146.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	24,426.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animals	45,000.00
9. Sequencing Core facility	25,000.00
10. Mitochondrial function / Synaptosome Core	8,000.00
11. MRI in mice	25,000.00
12. DOMSTAT	3,000.00
13. Tuition Fees	17,857.00
14. Technology Infrastructure Fees (TIF)	1,556.00
Total Other Direct Costs	**149,839.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**591,985.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research On Campus	56	574,128.00	321,512.00
		Total Indirect Costs	**321,512.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Janet Turner, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**913,497.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	913,497.00

L. Budget Justification*	File Name:
	Budget_Justification_RV1071243866.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2026 **End Date*:** 03-31-2027 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Rhonda	R.	Voskuhl	MD	PD/PI	203,700.00	6.6			112,035.00	43,133.00	155,168.00
2 .	Dr.	Yuichiro		Itoh	PhD	Co-Investigator	107,900.00	4.2			37,765.00	18,240.00	56,005.00
3 .	Dr.	Jin		Zhou	PhD	Co-Investigator	160,000.00	0.6			8,000.00	3,080.00	11,080.00
4 .	Dr.	Allan		MacKenzie-Graham	PhD	Co-Investigator	137,900.00	1.8			20,685.00	7,964.00	28,649.00
5 .	Dr.	Prabha		Siddarth	PhD	Co-Investigator	153,697.00	0.6			7,685.00	3,712.00	11,397.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **262,299.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6			36,971.00	1,479.00	38,450.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Lab Technician	12			74,500.00	40,081.00	114,581.00
1	MRI Lab Technician	3.6			15,485.00	8,331.00	23,816.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**176,847.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**439,146.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT13677549 Funding Opportunity Number: RFA-NS-22-038 . Received Date: 2022-07-08T17:54:10.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2026 **End Date*:** 03-31-2027 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2026 **End Date*:** 03-31-2027 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	24,426.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animals	45,000.00
9. Sequencing Core facility	25,000.00
10. Mitochondrial function / Synaptosome Core	8,000.00
11. MRI in mice	25,000.00
12. DOMSTAT	3,000.00
13. Tuition Fees	17,857.00
14. Technology Infrastructure Fees (TIF)	1,556.00
Total Other Direct Costs	**149,839.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**591,985.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research On Campus	56	574,128.00	321,512.00
		Total Indirect Costs	**321,512.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Janet Turner, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**913,497.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	913,497.00

L. Budget Justification*	File Name:
	Budget_Justification_RV1071243866.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2027 **End Date*:** 03-31-2028 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Rhonda	R.	Voskuhl	MD	PD/PI	203,700.00	6.6			112,035.00	43,133.00	155,168.00
2 .	Dr.	Yuichiro		Itoh	PhD	Co-Investigator	107,900.00	4.2			37,765.00	18,240.00	56,005.00
3 .	Dr.	Jin		Zhou	PhD	Co-Investigator	160,000.00	0.6			8,000.00	3,080.00	11,080.00
4 .	Dr.	Allan		MacKenzie-Graham	PhD	Co-Investigator	137,900.00	1.8			20,685.00	7,964.00	28,649.00
5 .	Dr.	Prabha		Siddarth	PhD	Co-Investigator	153,697.00	0.6			7,685.00	3,712.00	11,397.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **262,299.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6			36,971.00	1,479.00	38,450.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Lab Technician	12			74,500.00	40,081.00	114,581.00
1	MRI Lab Technician	3.6			15,485.00	8,331.00	23,816.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**176,847.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**439,146.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2027 **End Date*:** 03-31-2028 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT13677549 Funding Opportunity Number: RFA-NS-22-038 . Received Date: 2022-07-08T17:54:10.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2027 **End Date*:** 03-31-2028 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	24,426.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animals	45,000.00
9. Sequencing Core facility	25,000.00
10. Mitochondrial function / Synaptosome Core	8,000.00
11. MRI in mice	25,000.00
12. DOMSTAT	3,000.00
13. Tuition Fees	17,857.00
14. Technology Infrastructure Fees (TIF)	1,556.00
Total Other Direct Costs	**149,839.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**591,985.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research On Campus	56	574,128.00	321,512.00
		Total Indirect Costs	**321,512.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Janet Turner, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**913,497.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	913,497.00

L. Budget Justification*	File Name:
	Budget_Justification_RV1071243866.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 6

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2028 **End Date*:** 03-31-2029 **Budget Period: 6**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Rhonda	R.	Voskuhl	MD	PD/PI	203,700.00	6.6			112,035.00	43,133.00	155,168.00
2 .	Dr.	Yuichiro		Itoh	PhD	Co-Investigator	107,900.00	4.2			37,765.00	18,240.00	56,005.00
3 .	Dr.	Jin		Zhou	PhD	Co-Investigator	160,000.00	0.6			8,000.00	3,080.00	11,080.00
4 .	Dr.	Allan		MacKenzie-Graham	PhD	Co-Investigator	137,900.00	1.8			20,685.00	7,964.00	28,649.00
5 .	Dr.	Prabha		Siddarth	PhD	Co-Investigator	153,697.00	0.6			7,685.00	3,712.00	11,397.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **262,299.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6			36,971.00	1,479.00	38,450.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Lab Technician	12			74,500.00	40,081.00	114,581.00
1	MRI Lab Technician	3.6			15,485.00	8,331.00	23,816.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**176,847.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**439,146.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 6

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2028 **End Date*:** 03-31-2029 **Budget Period: 6**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 6

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2028 **End Date*:** 03-31-2029 **Budget Period: 6**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	24,426.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animals	45,000.00
9. Sequencing Core facility	25,000.00
10. Mitochondrial function / Synaptosome Core	8,000.00
11. MRI in mice	25,000.00
12. DOMSTAT	3,000.00
13. Tuition Fees	17,857.00
14. Technology Infrastructure Fees (TIF)	1,556.00
Total Other Direct Costs	**149,839.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**591,985.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research On Campus	56	574,128.00	321,512.00
		Total Indirect Costs	**321,512.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Janet Turner, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**913,497.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	913,497.00

L. Budget Justification*	File Name:
	Budget_Justification_RV1071243866.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 7

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2029 **End Date*:** 03-31-2030 **Budget Period: 7**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Rhonda	R.	Voskuhl	MD	PD/PI	203,700.00	6.6			112,035.00	43,133.00	155,168.00
2 .	Dr.	Yuichiro		Itoh	PhD	Co-Investigator	107,900.00	4.2			37,765.00	18,240.00	56,005.00
3 .	Dr.	Jin		Zhou	PhD	Co-Investigator	160,000.00	0.6			8,000.00	3,080.00	11,080.00
4 .	Dr.	Allan		MacKenzie-Graham	PhD	Co-Investigator	137,900.00	1.8			20,685.00	7,964.00	28,649.00
5 .	Dr.	Prabha		Siddarth	PhD	Co-Investigator	153,697.00	0.6			7,685.00	3,712.00	11,397.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **262,299.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6			36,971.00	1,479.00	38,450.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Lab Technician	12			74,500.00	40,081.00	114,581.00
1	MRI Lab Technician	3.6			15,485.00	8,331.00	23,816.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**176,847.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**439,146.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-NS-22-038 . Received Date: 2022-07-08T17:54:10.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 7

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2029 **End Date*:** 03-31-2030 **Budget Period: 7**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT13677549 Funding Opportunity Number: RFA-NS-22-038 . Received Date: 2022-07-08T17:54:10.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 7

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2029 **End Date*:** 03-31-2030 **Budget Period: 7**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	24,426.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animals	45,000.00
9. Sequencing Core facility	25,000.00
10. Mitochondrial function / Synaptosome Core	8,000.00
11. MRI in mice	25,000.00
12. DOMSTAT	3,000.00
13. Tuition Fees	17,857.00
14. Technology Infrastructure Fees (TIF)	1,556.00
Total Other Direct Costs	**149,839.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**591,985.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research On Campus	56	574,128.00	321,512.00
		Total Indirect Costs	**321,512.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Janet Turner, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**913,497.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	913,497.00

L. Budget Justification*	File Name:
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 8

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2030 **End Date*:** 03-31-2031 **Budget Period: 8**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
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3 .	Dr.	Jin		Zhou	PhD	Co-Investigator	160,000.00	0.6			8,000.00	3,080.00	11,080.00
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Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **262,299.00**

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Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6			36,971.00	1,479.00	38,450.00
	Undergraduate Students						
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						Total Salary, Wages and Fringe Benefits (A+B)	**439,146.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 8

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2030 **End Date*:** 03-31-2031 **Budget Period: 8**

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Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

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2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 8

UEI*: RN64EPNH8JC6

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the University of California, Los Angeles

Start Date*: 04-01-2030 **End Date*:** 03-31-2031 **Budget Period: 8**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	24,426.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animals	45,000.00
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Total Other Direct Costs	**149,839.00**

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Total Direct and Indirect Institutional Costs (G + H)	**913,497.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	913,497.00

L. Budget Justification*	File Name:
	Budget_Justification_RV1071243866.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

The annual direct costs requested in this R35 were calculated by Dr. Alisa Schaefer, NINDS, NIH, on June 2, 2021 based on the 4-year average of direct costs of my current award numbers R01NS096748 and R01NS109670 multiplied by 1.2. The annual direct cost for this R35 is $591,985.50 per year.

FY	PI Name	Type	Project Num	Awd Dir Cost	FY 2017	FY 2018	FY 2019	FY 2020	FY 2021	average FY18-21	average * 1.2
2022	VOSKUHL, RHONDA R	5	R01NS109670-05	$284,984	$277,783	$562,767	$562,767	$562,767	$284,984	$493,321.25	$591,985.50
2021	VOSKUHL, RHONDA R	5	R01NS109670-04	$284,984							
2020	VOSKUHL, RHONDA R	5	R01NS096748-05	$277,783							
2020	VOSKUHL, RHONDA R		R01NS109670-03	$284,984							
2019	VOSKUHL, RHONDA R	5	R01NS096748-04	$277,783							
2019	VOSKUHL, RHONDA R		R01NS109670-02	$284,984							
2018	VOSKUHL, RHONDA R	1	R01NS109670-01	$284,984							
2018	VOSKUHL, RHONDA R	5	R01NS096748-03	$277,783							
2017	VOSKUHL, RHONDA R	5	R01NS096748-02	$277,783							

PERSONNEL

Actual salaries (or NIH cap) and UCLA's composite benefit rate were utilized in this multi-year project.

SENIOR/KEY PERSONNEL

Rhonda Voskuhl, M.D., Principal Investigator (6.60 calendar months each year). Dr. Voskuhl will be responsible for research direction which drives the selection of the production of mice of all genotypes, MS disease models, and overall organization. All basic mouse experiments are considered in the context of MS clinical observations and unmet need. In addition, Dr. Voskuhl will use human MS imaging, transcriptomics and immunohistochemistry to guide and prioritizes work in mouse models. Within preclinical work, Dr. Voskuhl will apply the results of changes in gene expression primarily using chronic EAE to identify cell-specific, region-specific and sex-specific targets. She will employ the cuprizone model as needed depending on the question. She will be primarily responsible for directing experimental implementation as well as coordinating interpretation of transcriptomic results for prioritization of mechanistic studies to pursue. This will include experimental planning, analyses, and progress to publications. She will directly oversee work by her senior lab technician, Ms. Noriko Itoh, in charge of immunohistochemistry and will work closely with the graduate student on both scientific issues and career development goals. Dr. Voskuhl will assure that administrative issues are managed, that deadlines are met (e.g., regulatory approvals, findings properly disseminated to the scientific community), and that fiscal matters are properly executed. She will direct the trajectory of the research and be responsible for writing of manuscripts with assistance from collaborators.

Yuichiro Itoh, Ph.D. Co-Investigator (4.20 calendar months each year). Dr. Itoh will oversee breeding strategies and generation of cohorts of CNS cell-specific RiboTag mice and selective deletion of conditional knockouts in each CNS cell. He will get RNA sequencing data from the UCLA Sequencing Core and proceed to do RNA sequencing and gene expression pathway enrichment analyses. He will also design mice for epigenetic studies. He has carried out these studies in our publications. Dr. Itoh will also be in charge of single nuclei RNA sequencing as well as analyses in human MS snRNA-seq data (in collaboration with Dr. Jin Zhou of DOMStat). Results in human MS will guide preclinical work on top differentially expressed gene pathways in EAE for genetic and pharmacologic knock out and knock down, respectively. Dr. Itoh will work closely with Dr. Voskuhl and Dr. Zhou during these analyses regarding interpretation and further experimentation. Dr. Itoh will also assist with writing of manuscripts particularly in the area of gene expression and pathway analyses.

Jin Zhou, Ph.D., Co-Investigator (0.6 calendar months each year). Associate Professor, Dept of Medicine Statistics Core (DOMStat). Dr. Zhou will carry out transcriptomics analyses which integrate gene expression data across CNS cell types, CNS regions, both sexes, and across aging stages. She will assess interactions between these variables within the mouse data. Dr. Zhou will also be in charge of methylation statistical analyses, as well as integrating methylome data with transcriptome data. Dr. Zhou will mine existing human scRNA-seq datasets (in collaboration with Dr. Itoh) to help guide further mouse studies. She will work with Dr. Siddarth to integrate transcriptomics data with region-specific cellular and regional brain volume data. She has full access to facilities in the DOMStat Core (see Letter of Support from DOMStat Director, Dr. David Elashoff).

Allan MacKenzie-Graham, Ph.D. Co-Investigator (1.8 calendar months each year). Dr. MacKenzie-Graham will

be in charge of all neuroimaging acquisition, analysis, and interpretation (MRI). As faculty of the UCLA Brain Mapping Center, he has full access to the dedicated small animal MRI scanner. He will directly oversee work by the part time MRI neuroimaging technician. Dr. MacKenzie-Graham will provide neuroimaging data for Dr. Siddarth, for her to do statistical analyses correlating changes in gene expression with changes in localized gray matter atrophy. Dr. MacKenzie-Graham will also be in charge of CLARITY analysis for 3D neuropathology. Clarity neuropathology will also be correlated with localized gray matter atrophy. This will be done in collaboration with Dr. Voskuhl (PI) and with Dr. Siddarth. Dr. MacKenzie-Graham and Dr. Voskuhl have an ongoing collaboration investigating region-specific and sex-specific substructure atrophy using human MS datasets sent from Dr. Freidemannn Paul's group at Charite, Univ. of Berlin to UCLA. Dr. MacKenzie-Graham will lead the human image analyses, while working with Dr. Voskuhl and Dr. Paul. He will assist with writing of manuscripts particularly in the area of neuroimaging.

Prabha Siddarth, Ph.D. Co-Investigator (0.6 calendar months each year). Senior Statistician, UCLA Semel Institute Statistics Core (SiStat). Dr. Siddarth will carry out statistical analyses between quantitative expression of key genes of focus at the protein level by immunohistochemistry in each region with corresponding regional substructure brain volumes, in each sex and at each age. This will include regressions, multimodality correlations, and predictive probabilities. It will also include between groups comparisons by age, sex, and age by sex interactions. Dr. Siddarth will work with Dr. Zhou as they integrate gene neuropathology and neuroimaging statistics with transcriptomics statistics. As Semel Institute faculty, she has full access to the UCLA Semel Institute Statistics Core (SiStat). She will also assist with writing of manuscripts particularly in the area of statistical analyses.

This team of investigators has an extensive track record of successful collaboration with many shared publications and grants over the last several years.

OTHER PERSONNEL

Graduate student (GSR), 6.0 calendar months each year). The graduate student (Diego Cortez-Delgado currently) with work closely with Dr. Voskuhl to be mentored in cell-specific, region-specific, and sex-specific research using transcriptomics followed by determination of causality through gene deletion in the cell identified by gene expression pathway analysis. This trainee will learn hands on lab techniques from Ms. Itoh, and is expected to become increasingly independent. He will learn genetic engineering mouse design from Dr. Itoh and transcriptome analyses from Dr. Itoh and Dr. Zhou. The graduate student will be heavily involved in manuscript writing and will apply for graduate student fellowships based on the project. Notably, the overall project is highly ambitious, requiring Ms. Itoh and this GSR to focus on different cell types, pathways, sex differences, and aging aspects. This creates parallel work which is well supervised. Upon attaining his degree, this GSR will be replaced by another during the planned 8 year duration of this award.

Noriko Itoh, M.S., Senior Lab Technician, (12.0 calendar months each year). Under the supervision of Dr. Voskuhl, Ms. Itoh will be responsible for breeding colonies, genotyping, EAE induction, and treatment. She will sacrifice and perfuse all mice for collection of CNS tissues. Ms. Itoh will be responsible for tissue acquisition, obtaining RNAs from cell specific RiboTag mice, validation of HA labeling and cell-specific enrichment, as well as validation of changes in gene expression by RT-qPCR and immunohistochemistry. She will also do clinical EAE scoring and EAE neuropathology as well as mitochondrial & synaptosome experiments in the dedicated UCLA core. She will provide care to EAE mice as per veterinarian recommendations and ARC guidelines. Ms. Itoh maintain ARC for approvals and continuations.

MRI Lab Technician, (3.6 calendar months each year). Under the supervision of Dr. MacKenzie-Graham, the MRI lab technician will acquire MRI scans of all cohorts of mice in the Brain Mapping Center, then carry out image processing and analyses of all raw image data for localized gray matter atrophy using atlas-based morphometry under his direction.

Benefit rates are calculated at 38.5% for HCOMP Faculty, 48.3% for Other Academics, 4% for GSR, and 53.8% for Non-exempt. The fringe benefit rates used have been proposed to DHHS.

Travel

Domestic travel of two investigators to scientific conference each year (within the U.S.). $3,000 per year.Travel

costs for each: $750 for airfare, $600 for three nights lodging, and $150 for food and taxi.

Other Costs

Lab reagents: $24,426 per year
This includes funds for RNA isolations, PCR for genotyping, qPCRs for expression analysis at the RNA level, antibodies for immunohistochemistry, EAE induction, EAE and placebo treatments.

Animals: DLAM (mouse vivarium): $45,000 per year
Extensive mouse colonies will be needed to support the study of transcriptomics in astrocytes, oligodendrocytes, microglia, and neurons, in both females and males, and at different life stages. Also double-label transcriptomics in 2 cells within the same mice and cell-specific gene deletion mice will be done.
Sequencing Core facility: $25,000 per year
Extensive RNA sequencing will be needed to determine gene expression in astrocytes, oligodendrocytes, microglia, and neurons, in both females and males, and at different life stages during disease as compared to healthy controls.

Mitochondrial function / Synaptosome Core: $8,000 per year
Core services are needed for respirometry assays by SeaHorse Analyzer as an outcome of mitochondrial function in neurons as a functional correlate of neuropathology.

MRI in mice: $25,000 per year

Atlas based morphometry analysis of in vivo MRIs in both females and males, and at different ages during EAEas compared to healthy controls will localize the region of gray matter atrophy and the impact of cell-specific knockouts and pharmacologic treatments.

DOMStat $3,000 per year: Core Data Management and statistical analysis staff to support Dr. Zhou and Dr. Siddarth.

Tuition Fees $17,857 per year: The university policy is graduate student researcher working at 25% or greater in a lab is eligible for tuition/remission fees. Funds are requested to offset UCLA tuition fees and health insurance costs for the listed graduate student. These funds will be used to warrant that the graduate student researcher can focus the efforts on this project.

Technology Infrastructure Fees (TIF): Funding is requested for teleconferences and telecommunications, as UCLA charges a technology infrastructure fee of $43.96/calendar month per employee for communications andtelephone services. TIF pays for campus communication services on the basis of a monthly accounting of actual usage data. TIF is a charge for campus network, backbone, internal connection, hardware, wireless services, etc. These mandatory costs are charged as direct costs and are not recovered as indirect costs. We are requesting $1,586 each year.

Facilities and Administrative Costs: Indirect Costs are based on the institutions federally negotiated agreement dated 10/12/2018. Effective 7/1/19 our rate becomes provisional. Awards using provisional rates must be adjusted once a new F&A rate agreement is negotiated and approved by the cognizant agency for indirect costs. MTDC is excluding student tuition/remission costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		2,098,392.00
Section B, Other Personnel		1,414,776.00
Total Number Other Personnel	24	
Total Salary, Wages and Fringe Benefits (A+B)		3,513,168.00
Section C, Equipment		
Section D, Travel		24,000.00
1. Domestic	24,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		1,198,712.00
1. Materials and Supplies	195,408.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	360,000.00	
9. Other 2	200,000.00	
10. Other 3	64,000.00	
11. Other 4	200,000.00	
12. Other 5	24,000.00	
13. Other 6	142,856.00	
14. Other 7	12,448.00	
15. Other 8		
16. Other 9		
17. Other 10		
Section G, Direct Costs (A thru F)		4,735,880.00
Section H, Indirect Costs		2,572,096.00

Section I, Total Direct and Indirect Costs (G + H)	7,307,976.00
Section J, Fee	
Section K, Total Costs and Fee (I + J)	7,307,976.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 09/30/2024

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ○ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 09/30/2024

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	
3. Research Strategy*	R35_Research_Strategy1071243917.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals1071122660.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	Letters_Of_Support1071243833.pdf
10. Resource Sharing Plan(s)	Resource_Sharing_Plan_R351071243743.pdf
11. Authentication of Key Biological and/or Chemical Resources	Auth_Key_Reagent_R351071243731.pdf
Appendix	
12. Appendix	

A new approach to a major unmet need. Multiple sclerosis (MS) is an autoimmune and neurodegenerative disease with inflammatory lesions, demyelination, axonal damage, glial activation and synaptic loss. There are acute relapses and accumulation of permanent disabilities. There are over 20 disease modifying treatments (DMTs) targeting cells and mechanisms in the immune system, with robust effects on relapses. There remains a need for DMTs that target cells in central nervous system (CNS) to halt neurodegeneration and repair disabilities. The CNS is a highly complex target organ of the autoimmune attack in MS. I hypothesize that neuroprotective treatments in MS will not work using a "one size fits all" approach using Phase 3 trials with a composite of disabilities (Expended Disabilities Status Scale, EDSS) and whole brain atrophy as outcomes. Three clinical observations are clues to a new approach: **1) MS patients are heterogenous regarding which disabilities are predominant, 2) being female versus male impacts disability worsening, and 3) aging aligns with disability progression.** We will use a cell-specific, region-specific, and sex-specific approach to discover optimal neurodegenerative treatment targets for distinct disabilities in MS women and men. Beyond this R35, future clinical trials in MS will target a specific disability as the primary outcome measure with corresponding regional MRI abnormalities as the biomarker, and enrollment will be tailored for sex and age.

Overview: Our preclinical work begins (clinical observations) and ends (clinical trials) with MS patients, **Fig. 1.** Also, at the laboratory bench, human MS data guide preclinical lead prioritization and provide validation at three steps (Fig. 1, asterisks). This R35 will 1) Extend our recent work in astrocytes (1-3) and oligodendrocytes (4, 5) to microglia and neurons, with cell:cell interactions revealed in mice double-labelled to study gene expression changes in two distinct cell types in the same region in the same mouse, and 2) Determine if there is an effect of **sex** and/or **age** on the most differentially expressed cell-specific and region-specific pathways. This R35 takes our research to the next level: Identifying sex by age interactions in cell-specific and region-specific transcriptomics, neuropathology, and regional atrophy on MRI.

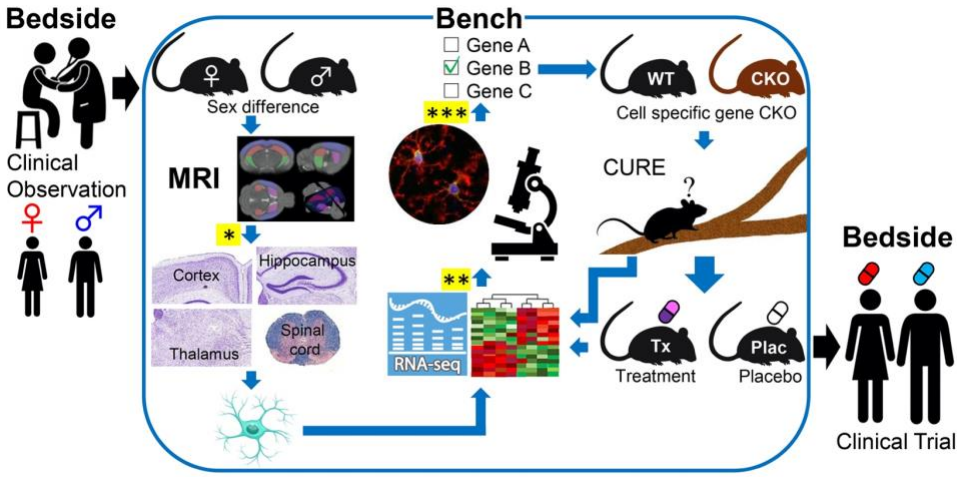

Fig. 1. Bedside to Bench to Bedside research: A region-specific, cell-specific, and sex-specific approach to neurodegeneration in MS. Clinical observations of sex differences are investigated at the preclinical level then translated back to the clinic as trials designed for each sex. Bench investigations entail *in vivo* MRI for region-specific atrophy, neuropathology of each region, RNA-sequencing of each CNS cell from each region, immunohistochemistry validation of top genes in highly differentially expressed pathways, conditional knockout (CKO) of target genes in each CNS cell to reverse phenotype, and knockdown of target genes with pharmacologic treatment (Tx) to reverse phenotype. The effect of genetic (CKO vs WT) and/or pharmacologic (treatment vs placebo) intervention on reversal of gene expression is determined using the same cell-specific and region-specific approach in each sex.
Human MS data guide preclinical research at 3 checkpoints (yellow highlighted asterisks, left of arrows):
* MRI in females and males with MS revealing sex differences in regions of atrophy will prioritize regions in EAE with atrophy.
** snRNA-seq analyses in females and males with MS revealing gene pathways of interest will prioritize gene pathways in EAE.
*** immunohistochemistry in females and males using MS postmortem tissues will validate immunohistochemistry in EAE.
Informative substitutions to the use of female versus male mice in the beginning (upper left), include: i) use of gonadectomized versus gonadally intact mice to reveal activational effects of sex hormones; ii) use of Four Core Genotype mice to reveal sex chromosome effects versus developmental sex hormone effects; and iii) use of young versus old mice to reveal the effect of aging. Use of more than one of these interventions in the same experiment will reveal sex hormone by age interactions or sex chromosome by age interactions.

A cell-specific and region-specific approach: Premise. Our disability-specific approach is based on the heterogeneity of disabilities in MS patients, and that distinct disabilities (walking, vision, cognition, coordination) align with different CNS regions. Even in healthy brain, a given CNS cell type differs in gene expression from one brain region to another for neurons (6), microglia (7), astrocytes (8, 9), and oligodendrocytes (10, 11). Mechanisms driving neurodegeneration underlying distinct disabilities in MS are unlikely to be identical across the CNS. So too, treatments to reverse neurodegeneration are unlikely to be identical across the CNS.

My lab has extensive experience in determining CNS cell-specific and region-specific gene expression changes in MS preclinical models (1, 2, 4, 12). We hypothesized that there would be regional differences in gene expression in response to injury during neurodegenerative disease and were the first to show it in chronic experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice (2). To avoid effects of *in vitro* cell isolation

on gene expression (13), we used *in vivo* RiboTag technology (14). Our cell-specific and region-specific transcriptomics approach identified the following candidate neuroprotective treatment targets: Spinal cord astrocytes have decreased expression of cholesterol synthesis pathway genes during EAE. In adults, cholesterol is synthesized by astrocytes and transported out via ATP-binding cassette transporter (ABCA1) to apolipoprotein E (ApoE) (15) to oligodendrocytes to make myelin (15, 16) to neurons for synaptic plasticity (17, 18). While damage in EAE is due to the autoimmune attack, we posited that failure of remyelination and synaptic plasticity is due to decreased cholesterol synthesis in astrocytes (2). Treatment with an ABCA1 agonist improved walking and reduced spinal cord pathology (2). This was confirmed by others (19). Optic nerve astrocytes have increased expression of complement pathway genes during optic neuritis in EAE (1), which we validated in MS using human postmortem tissues (2). This suggested targeting complement in optic neuritis in MS (1), which was confirmed by others (20). Also, higher C3 expression in females (1) suggested potential sex differences in efficacy of treatments targeting complement (21). Corpus callosum oligodendrocytes have increased expression of cholesterol synthesis pathway genes in oligodendrocytes during remyelination (4). Estrogen receptor beta (ERβ) ligand treatment recapitulated the promyelinating effect of estriol in the blood of pregnant mothers. It increased cholesterol synthesis gene expression in oligodendrocytes and enhanced remyelination in cuprizone & EAE models (4, 5). $CD4^+$ T lymphocytes: *Kdm6a*, an X chromosome gene, increased neuroinflammatory pathway signaling in $CD4^+$ T cells. Knockout of *Kdm6a* in $CD4^+$ T cells reversed the transcriptome and ameliorated EAE (22). **Studies in MS tissues have guided our preclinical studies (2, 4, 22, 23).**

A sex-specific approach: Premise. Our sex-specific approach in MS is based on sex differences in MS. Females are more susceptible to MS (24-27), but males show faster disability progression and worse gray matter atrophy (24, 26, 28-33). Sex differences in the healthy brain exist from mice to humans (34-39), providing evidence for effects of sex hormones and/or sex chromosomes. There are sex differences in substructure volumes even when accounting for differences in brain size (34, 36-38, 40-48). There are also sex differences in the brain at the cellular, molecular, and functional levels (49-54). Thus, mechanisms of neurodegeneration are unlikely to be identical in females and males. The study of sex differences capitalizes on a clinical observation, disentangling it at the laboratory bench, then translating findings to the clinic as a novel treatment trial (26). The importance of sex as a biologic variable (SABV) has been recognized by the NIH (55, 56). In this R35, identifying the effect of sex on the most differentially expressed cell-specific and region-specific pathways can lead to disability-specific neuroprotective treatments tailored for each sex.

I am a leader in the study of sex differences in the immune system and the CNS (26, 32, 33). Like other autoimmune diseases with a female bias, MS susceptibility is thought to be due to sex differences in immune responses (57, 58). We disentangled sex hormone and sex chromosome effects in EAE using the Four Core Genotypes (FCG) model to compare XX versus XY sex chromosome complements in the absence of the confound of a difference in sex hormones (59-61). We then determined whether differences in XX versus XY are due to X-gene dosage effects, parental imprinting of X genes, or Y gene effects (22, 62, 63). We also showed how sex hormone and sex chromosome effects can be complementary or compensatory in function (59, 64). For example, in EAE the XX genotype is proinflammatory in T lymphocytes, while estrogens at a level and type consistent with pregnancy are neuroprotective (26, 32, 65). This neuroprotection in EAE extends beyond spinal cord to include cerebral cortex, hippocampus and cerebellum (5, 66-68), and it is dependent on dose, timing, estrogen type (estradiol, estriol), and estrogen receptor (ER) ligation (ERα, ERβ) (5, 32, 66, 69, 70).

Sex differences in neurodegeneration during aging. Gray matter atrophy and disability progression are worse in MS men from young adulthood to midlife, median age < 50 years (71-73). In contrast, older women have worsening of their MS disabilities after menopause (74-80). Loss of neuroprotective estradiol with menopause is consistent with cognitive difficulties in healthy women with menopause, quantified by objective cognitive testing (81-87) and termed “brain fog” (81, 82, 84, 88-91). Alzheimer’s disease (AD) is more common in females, which is not accounted for by longevity (92-94). Women ages > 60 years have a higher rate of progression from Mild Cognitive Impairment (MCI) to AD and faster rates of brain atrophy. In contrast, at ages < 60 years, women are not at higher risk for MCI, and men have higher rates of progression from MCI to AD (94). Loss of sex hormones during menopause and andropause aligns with cognitive decline and increased AD risk (95-97). Similar negative effects of menopause and andropause may be due to testosterone’s conversion to estradiol in brain by aromatase. Decreased levels of either hormone during aging reduces estrogen receptor ligation in brain (98). In MS men, worse cognitive decline correlates with lower testosterone levels (99), and testosterone treatment reduced brain atrophy in a pilot trial by the PI (100, 101). In a placebo-controlled clinical trial by the PI (69), treatment with estriol, a natural ERβ ligand, reduced cerebral cortex atrophy and improved cognition (69, 102).

Cognitive improvement correlated with cerebral cortex sparing and estriol blood level. Serum neurofilament light chain (sNfL) levels were decreased by estriol treatment, and this correlated with less neuroaxonal injury (103).

Sex by age interactions: Hypothesis. Aging is a risk factor for the transition from the inflammatory MS phase to the neurodegenerative phase (28, 104). During health in mice and humans, aging aligns with brain atrophy, neurodegeneration, and cognitive decline. **I hypothesize that the effect of biologic sex during the lifespan is complex, with sex hormones and sex chromosomes contributing differently based on the timing of the loss of neuroprotective sex hormones during andropause versus menopause.** Andropause starts at age 30 with gradual decline of testosterone to old age (Fig. 2, blue). Menopause starts later (ages 46-52) with an abrupt decline in hormones (Fig. 2, red). At 75 to 90 years, loss of neuroprotective sex hormones in both sexes may unmask underlying effects of sex chromosomes (XX vs XY) that persist across the lifespan (Fig. 2, green). Here, we will determine whether sex differences in cell-specific and region-specific gene expression during aging are due to effects of sex hormones and/or sex chromosomes and identify the hormone receptor and/or sex chromosome gene responsible.

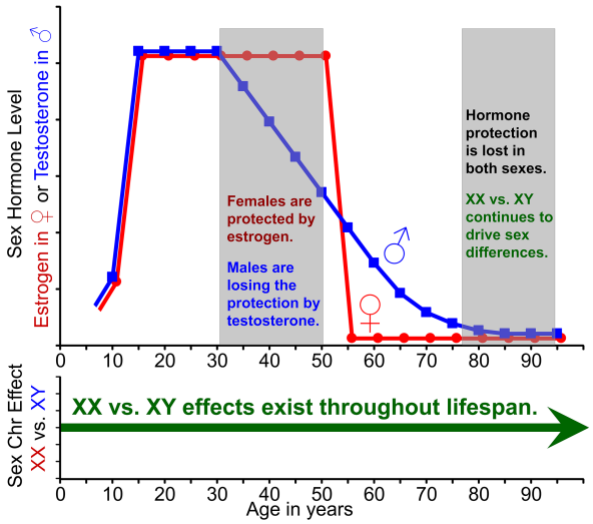

Fig. 2. Sex hormone and sex chromosome effects during aging: Neurodegeneration in males is worse before midlife, with females worse after midlife.

Preliminary data: A sex hormone by age interaction. Understanding the effect of brain aging during health can provide insights into the effect of brain aging during MS. Healthy menopausal women experience cognitive difficulties at menopause. Treatments are needed that target cognition in healthy menopausal women and target disability worsening in MS menopausal women. We recently discovered a sex difference in brain substructure atrophy in healthy aging mice by *in vivo* MRI (Fig. 3) and found a sex difference in the effect of gonadectomy on cognitive function at midlife (Fig. 4a). Ovariectomy induced cognitive deficits (Fig. 4b), astrocyte activation (Fig. 4c), synaptic loss and dorsal hippocampal atrophy in females at midlife, but not young age. Deletion of ERβ in astrocytes in midlife females recapitulated the effect of ovariectomy on cognitive deficits and dorsal hippocampal atrophy (Fig 5). Thus, ligation of ERβ in astrocytes was identified as a target to prevent hippocampal-dependent cognitive deficits and dorsal hippocampal atrophy in healthy females at midlife (3).

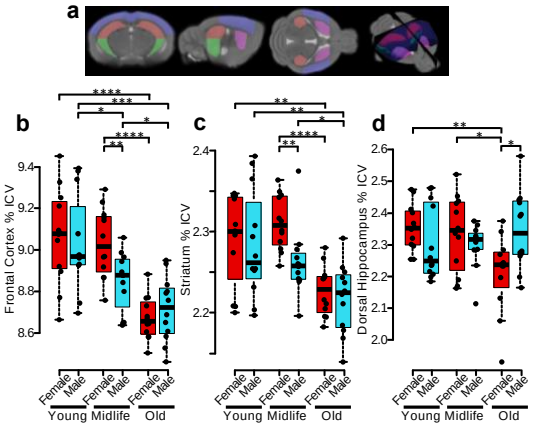

Fig. 3. In contrast to males (blue) who have gradual frontal cortex and striatum volume loss with aging, females (red) have substructure volume preservation from young age (3-4 months) to midlife (12-14 mo), followed by abrupt volume loss from midlife to old age (20-22 mo). a) substructure delineations; volumes expressed as % intracranial volume (ICV) in **b)** frontal cortex, **c)** striatum, **d)** dorsal hippocampus. red (females), blue (males). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

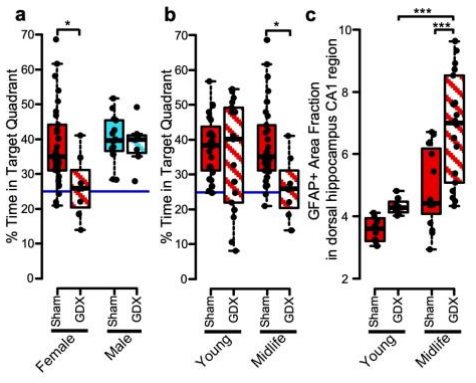

Fig. 4. Gonadectomy of females, but not males, worsens hippocampal-dependent cognition and increases astrocyte activation at midlife (12-14 mo), but not at young (3-4 mo) ages, revealing a sex hormone by age interaction. a,b) % time in target quadrant (Morris Water Maze); **c)** reactive astrocytosis (GFAP staining). red (females), blue (males); gonadally intact (solid), GDX (stripe).* $p < 0.05$; ** $p < 0.01$.

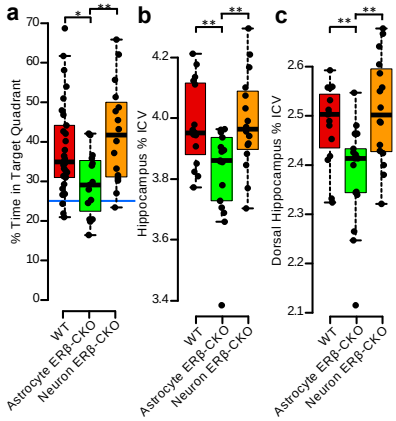

Fig. 5. Selective deletion of ERβ in astrocytes, but not neurons, induces hippocampal-dependent cognitive impairment and dorsal hippocampus atrophy at midlife in gonadally intact females. a) % time in TQ, **b)** whole hippocampal volumes, **c)** dorsal hippocampal volumes. red (WT), green (astrocyte ERβ CKO), orange (neuron ERβ CKO). * $p < 0.05$; ** $p < 0.01$.

We and others find that aging mice have worse walking and microglia activation in spinal cord during active and adoptive EAE (105). Here, we will identify age by EAE interactions in microglia (and astrocytes) in cord and also hippocampus, cerebral cortex, striatum, and cerebellum in each sex to find optimal targets.

Preliminary data: X chromosome gene. *Kdm6a* is a gene on the X chromosome that escapes X-inactivation and is expressed higher in XX (females) than XY (males) (22). KDM6A regulates expression of autosomal and sex chromosome genes by encoding for a histone demethylase that removes repressive trimethylation on histone H3 lysine 27 (H3K27me3) to expose chromatin for transcription (106, 107). When we selectively deleted *Kdm6a* in $CD4^+$ T lymphocytes, it reduced walking disability and neuropathology in spinal cord. The transcriptome in the CKO showed downregulation of expression of Neuroinflammation Signaling Pathway genes in $CD4^+$ T lymphocytes (22). This demonstrated that *Kdm6a* is proinflammatory in $CD4^+$ T lymphocytes during EAE, consistent with XX dosage increasing susceptibility of females to MS. Here, we will extend our approach to microglia. To make microglia-specific *Kdm6a* knockout mice, $Kdm6a^{f/f}$ (floxed/floxed) mice were crossed with *Tmem119*-Cre/ERT2 mice, and tamoxifen drove gene deletion. Data in *Tmem119*-Cre:RiboTag mice validated HA labelling specifically in microglia (Fig. 6a,b), and the microglial *Kdm6a* CKO showed increased H3K27me3 levels in microglia (Fig. 6c,d). Selective deletion of *Kdm6a* in microglia ameliorated clinical EAE in C57BL/6 mice (Fig. 6, right). Here, the effect of deletion of *Kdm6a* in microglia will be determined for pathology in spinal cord, hippocampus, cerebral cortex, striatum, cerebellum, and optic nerve. Whether substructure atrophy is reduced will be ascertained. The effect of the *Kdm6a* CKO on the microglial transcriptome will be revealed. Prioritization of validation of RNA-Seq candidates will be guided by analyses of data from human MS postmortem tissues examining the microglia transcriptome. The deleterious effect of *Kdm6a* in T cells and microglia in EAE contrasts with a report that *Kdm6a* is protective in the human amyloid precursor protein (hAPP) mouse model of Alzheimer's disease (108). Different outcomes may be due to a deleterious effect *Kdm6a* in the MS model versus a protective effect of *Kdm6a* in the AD model. Alternatively, differences may be due to targeting *Kdm6a* in microglia in spinal cord in MS versus neurons in hippocampus in AD. This underscores the importance of using a region-specific and sex-specific approach in each disease model.

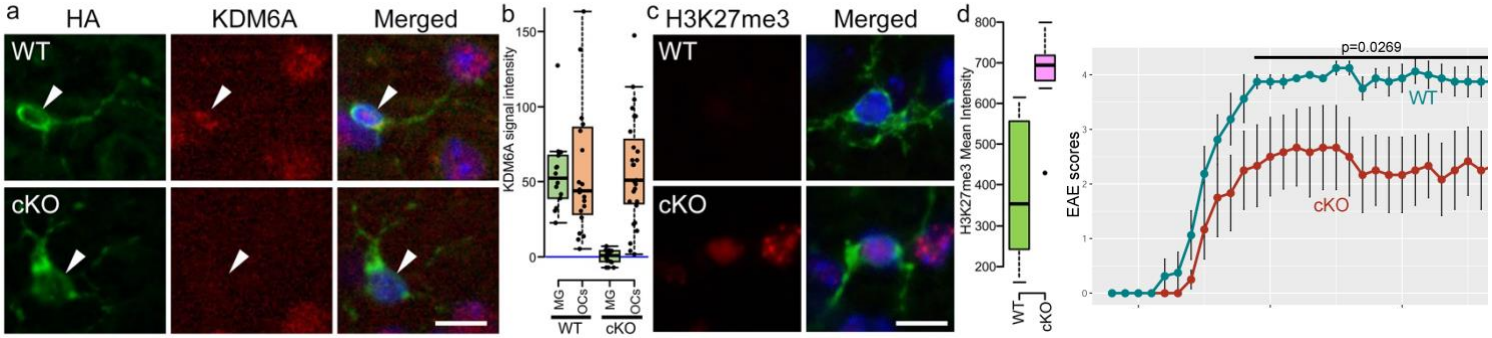

Fig. 6. Knockout of *Kdm6a* in microglia in *Tmem119*-Cre:RiboTag mice. In microglial *Kdm6a* cKO vs WT mice **a)** KDM6A was decreased. KDM6A (red), HA labelled microglia (green), DAPI (blue). **b)** quantification ($p < 0.0001$). **c)** H3K27me3 was increased in CKO. H3K27me3 (red), HA labelled microglia (green), DAPI (blue). **d)** quantification ($p < 0.001$). Tissues: dorsal hippocampus.
Right: *Kdm6a*-microglial-CKO mice have less severe EAE. $Kdm6a^{f/f}$-$Tmem119^{cre/ERT2+/-}$ (CKO) and $Kdm6a^{f/f}$-$Tmem119^{cre/ERT2-/-}$ (WT) mice were tamoxifen treated. (i.p. injection for 5 days). Eight weeks later, EAE was induced. CKO n=8, WT n=6 mice. All females. p-values, repeated measures ANOVA. The experiment was repeated, and again showed a significant CKO vs WT difference.

Details of Proposed Research Program in Fig. 1. We will identify treatment targets that are sex-specific, region-specific and cell-specific. Use of cell-specific RiboTag mice (14) on the C57BL/6 background permits deep sequencing of cells in the CNS in MS mouse models. Cell-specific gene deletion determines causality.
Preclinical models of MS: MS entails autoimmunity, CNS inflammation, glial activation, demyelination, and axonal damage, with minimal remyelination, in spinal cord, corpus callosum and optic nerve. There is also glial activation and synaptic damage in cerebral cortex, hippocampus, striatum, thalamus, and cerebellum, with substructure atrophy on MRI and retinal thinning on OCT. Our prior rationale for using chronic cuprizone treatment was to model the axonal damage and incomplete remyelination that occurs in MS (4), as opposed to lysolecithin or acute cuprizone models with minimal axonal damage and near complete remyelination. Chronic EAE (MOG 35-55 peptide in C57BL/6 mice) entails autoimmunity, CNS inflammation, demyelination, and axonal damage, with minimal remyelination in spinal cord (2, 5, 22, 109). It also affects corpus callosum (110-114) and optic nerve (1, 20, 115-127). Further, chronic EAE induces neuropathology in cerebral cortex (66, 109, 110, 128-133), hippocampus (67, 132, 134-147), striatum (141, 148-152), thalamus (153, 154), and cerebellum (66, 68, 128, 132, 155-157). Gray matter pathology in EAE entails microglial and astrocyte activation, synaptic loss and dysfunction, and substructure atrophy by MRI. Pathologies and atrophy progress over time from acute to chronic

EAE even after walking scores plateau at high values. MS patients also accumulate pathologies and atrophy after EDSS scores plateau at high values. EAE impairs walking, vision, cognition, and coordination, as it affects spinal cord, optic nerve, retina, hippocampus, cerebral cortex, cerebellum and striatum. **Preclinical models are critical to mechanism and discovery. There is never a perfect animal model for any human disease, including MS. The model used is based on the question asked. Acute, relapsing EAE is the most widely used model to develop anti-inflammatory DMTs in MS. Chronic EAE is a model of neurodegeneration in MS, with more than one neuropathology in more than one region, all in the context of autoimmunity.**

Principle Component Analysis, Differential Expression Analyses, and Canonical Pathway Analysis will identify the most differentially enriched pathways in chronic EAE, as described by our group (1, 2, 4, 22, 158). Single nuclei RNA sequencing (snRNA-seq) from human postmortem tissues (159), as well as analyses of existing human MS datasets (160-165), will be used to prioritize DE gene expression pathways in the preclinical model for validation and functional causality experiments. Retrieved snRNA-seq data will be analyzed accordingly to the protocol (e.g., 10x Genomics or CEL2-seq). We will use Seurat package (v.3.2.0 and v. 4.0.2.) in R for clustering and differential analysis. We will require genes to be expressed in more than five cells, as described (162). Additional QCs will be applied, such as the % of neuroinflammatory genes > 5%, and the number of expressed genes > 2500 (potentially “Multiplet”) will be removed prior to downstream analyses. A global-scaling normalization method will be employed, and gene expression will be log-transformed. Other normalization methods will also be considered for sensitivity analysis. Louvain clustering will be used to group cells, and cell type clusters will be identified using canonical marker genes. Non-linear dimensional reduction techniques, such as tSNE and UMAP, will be adopted to visualize and explore these datasets. Differentially expressed genes between MS and control will be identified using the FindMarkers function in Seurat, with non-parametric Wilcoxon rank-sum tests and adjustment for multiple comparison testing. Gene Ontology analyses (166) will be performed with AmiGO (Fisher Exact Test with FDR correction). Immunohistochemistry in postmortem tissues from MS will validate immunohistochemistry in EAE (2, 4, 23). MRI in MS women and men will reveal sex differences in regions of atrophy and prioritize regions of atrophy in EAE, as described, in collaboration with Dr. Friedemann Paul (33). Here, we will use an existing larger, longitudinal MS dataset (280 subjects followed for up to 5 years) from Charité, Universitätsmedizin Berlin (F. Paul-Letter of Support). Together, this will provide rationale for design of a future clinical trial of a DMT in MS to prevent substructure atrophy and improve the corresponding disability. See our publications on disability-specific atrophy in MS (33, 102, 167).

Our publications of cell-specific and region-specific gene expression in astrocytes and oligodendrocytes (1, 2, 4, 22) provide proof-of-principle that our approach can discover targets for neuroprotection. Here, we will extend this approach to microglia and neurons. We will compare females versus males to ascertain if there are sex differences in cell-specific and region-specific transcriptomes in EAE versus healthy mice. We will compare young adult (3-4 months), midlife (12-14 months), and old (20-22 months) mice in each sex. By integrating the different transcriptomic datasets, we will be able to perform comprehensive analyses permitting discovery of interactions between cells, regions, sex, and age. To ensure analysis reproducibility and rigor for transcriptomics data analyses, we will incorporate careful quality control of raw sequences, read alignment, and quantifying gene and transcript expression for differential expression analyses (168). DESeq2 (169), a count-based statistically powerful and computationally efficient tool, will be used for differential expression analysis between sexes. We will aggregate gene expression levels for genes in each pathway using principal component loadings. In addition, we will test for sex by cell, sex by region and age interactions to report cell-specific and region-specific effects of disease. Gene-level differential expression analysis will also be conducted with a false discovery rate (FDR) of 10%. Canonical pathway enrichment analysis will identify additional disease-related pathway enrichment. A transcriptomics database including data on cell type, region, sex, and age will be interrogated and hosted through Amazon Web Services (AWS) configured for UCLA Health Sciences. User interface will be implemented and deployed as a Shiny app for results dissemination. Our extensive publications showing sex hormone and sex chromosome effects in MS preclinical models demonstrate our ability to determine which X chromosome gene and which hormone/receptor causally impacts the disease-related transcriptome, neuropathology, and atrophy.

Cell-Cell Interactions. Here, we will determine the relationship between the transcriptomes of astrocytes and neurons within the same mouse. Aldh1l1-EGFP/Rpl10a mice (astrocytes) will be crossed with NSEII-Cre RiboTag mice (neurons) to create double-labelled mice (Aldh1l1-EGFP/Rpl10a: NSEII-Cre RiboTag), with immunoprecipitation of astrocyte- and neuron-specific RNAs using antibodies (anti-EGFP for astrocytes, anti-HA for neurons). We will determine which gene expression pathway changes in astrocytes correspond with changes in neurons during EAE by quantifying gene co-expressions of pathway pairs between astrocytes and neurons. Statistically efficient and computationally fast partial least square (PLS) regression will be adopted to evaluate the relationship between the two gene expression matrices for distinct pathways between the two cell

types (170). Statistical significance will be assessed by permutation test. We will control experiment-wide FDR at 10%. Sample size is based on preliminary data and number of comparisons. The same analysis will be conducted for two timepoints in EAE, early (day 12) and late (day 40), to reveal how cell-cell gene expression interactions evolve during disease. Tissues will be collected from 6 regions (motor cortex, hippocampus, striatum, cerebellum, optic nerve, and spinal cord) to determine regional differences in cell-cell interactions.

Not only can astrocytes modulate the neuronal synaptosome directly (8), they can also influence synapses indirectly though interactions of complement on astrocytes with complement receptor on microglia, which in turn affects phagocytosis of synapses (171, 172). We will cross Aldh1l1-EGFP/Rpl10a mice (astrocytes) with Tmem119-Cre RiboTag mice (microglia) to create double-labelled mice (Aldh1l1-EGFP/Rpl10a: Tmem119-Cre RiboTag mice). Immunoprecipitation of astrocyte and microglia specific RNAs will use antibodies (anti-EGFP for astrocytes, anti-HA for microglia) and RNA sequencing will reveal the transcriptome in each cell, in the same region. This will be done in EAE vs healthy controls. Statistical analyses (as above) will determine which gene expression pathway changes in astrocytes correspond with changes in microglia during early and late EAE.

Astrocyte-neuronal interactions and astrocyte-microglial interactions in each region will be contrasted between females versus males as well as between young (3-4 months) versus midlife (12-14 months) versus old (20-22 months). The effect of sex and age will be determined with modified query of the database, here accounting for the correlation measured within the same mouse. In particular, a random effect model (e.g., *duplicateCorrelation* function within limma package (173)) will be adopted to analyze repeated measured gene expressions. This will reveal how molecular signatures of these two cells change within a given mouse by region, in each sex, at each age, during EAE as compared to healthy controls. This will identify candidate treatments to halt or repair neurodegeneration within specific regions in each sex at the most optimal age.

Cross-modality correlations between neuropathology and neuroimaging. In a separate cohort of mice, *in vivo* brain MRIs will be collected (34, 128) and analyzed using atlas-based morphometry (ABM) to determine the degree of atrophy in cerebral cortex (109), hippocampus (134), striatum (109), and cerebellum (157, 174) during EAE. Imaged mice will undergo CLARITY for 3D neuropathology of intact tissues using the right half of brain (109, 175). MRI/CLARITY studies will use double transgenic mice (GFAP-TdTomato and Thy1-YFP) to show the relationship between astrocytes (GFAP) and synaptic density, dendritic arborizations, and neuronal number (Thy1). Co-registration of MRI data with CLARITY data from the same mice will reveal the neuropathology of astrocytes and neurons within regions of atrophy. Immunohistochemistry of sections of the other half of brain will assess microglial and astrocyte activation and synaptic density in gray matter, as well as glial activation, axonal damage and loss, and demyelination in white matter (176-178). Double-label immunohistochemistry will quantify expression of target genes identified by transcriptomics in the context of neuropathology. Relationships between regional atrophy and neuropathologies will entail regression analyses, as described (66, 68, 109, 128, 134, 157).

Epigenetics. To address mechanisms underlying gene expression changes, cell-specific epigenetics analysis (histone modifications and DNA methylation) will be done using Sun1-tagged INTACT mice (B6;129-Gt(ROSA)26Sor$^{tm5(CAG-Sun1/sfGFP)Nat}$/J) crossed with CNS cell-specific Cre mice. We will determine disease related epigenetics changes in WT mice as well as the effect of selectively deleting *Kdm6a* on H3K27me3 modification. Using methylSig (179), we will identify differentially methylated cytosines (DMCs) or regions (DMRs) of at least 15% change and control for FDR (10%), as we previously published (63). We will annotate DMCs and DMRs using UCSC Genome Browser's annotations for CpG islands, promoters, and other regions (180). For each DMCs and DMRs, we will calculate the correlation between DNA methylation and gene expression in the context of annotations (181). Notably, from INTACT mice, we will isolate 1) nuclear RNA for gene expression, 2) DNA for methylation, and 3) chromatin for histone modification.

Candidate treatments. Metformin occupies the same set of residues within the catalytic pocket of KDM6A which is involved in H3K27me3 binding and demethylation, thereby blocking KDM6A's effect on histone demethylase activity (107). Metformin has anti-aging properties (182-187) and protects in MS models (188-193). In contrast to effects on oligodendrocytes (188), we will focus on metformin's actions on *Kdm6a* in microglia (Fig. 1). While metformin is attractive due to repurposing, we will also test the more specific *Kdm6a* inhibitor, GSK-J4 (194). Our studies suggest that ERβ ligation in astrocytes prevents cognitive decline, dorsal hippocampal atrophy and neuropathology in healthy aging (3), while ERβ ligation in oligodendrocytes and CD11c^{+} cells induces remyelination in MS models (4, 5). The PI is an inventor on novel ERβ ligands patented and optioned by UCLA. Additional neurodegenerative targets will be discovered here in distinct CNS regions of females and males to generate additional candidate treatments optimal for distinct disabilities and tailored for MS women and men. **This R35 combines expertise in two RO1s, one using a region-specific, cell-specific approach (NS109670) and the other using a sex-specific approach (NS096748), to discover interactions. It also determines the effect of aging on these interactions. This body of work is ideally suited for an R35.**

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001
Expiration Date: 09/30/2024

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *	● Yes ❍ No
Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.	Human_Subjects1071243793.pdf
Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Other Requested Information	

Human Subjects:

No human subjects will be recruited, followed, or treated in this proposal.

Existing clinical and imaging data from human subjects of the University of Berlin were previously de-identified and transferred to UCLA. Proposed experiments will examine this existing dataset in place currently at UCLA.

Human postmortem tissues have been previously used in this group's publications. The tissues are de-identified and no additional informed consent is necessary for their use. Over the course of the 8 year R35, publicly available postmortem tissue datasets will be used.

Vertebrate Animals Section

1. Description of Procedures

Provide a concise description of the proposed procedures to be used that involve vertebrate animals. Identify the species, strains, ages, sex, and total number of animals by species to be used. If dogs or cats are proposed, provide the source of the animals. Note, for applications with due dates on or after May 25, 2016: (1) the method of euthanasia is eliminated from the VAS and is addressed in the FORMS-D Cover Page Supplement or PHS Fellowship Supplemental forms; (2) a description of veterinary care is no longer required; and (3) the justification for the number of animals has been eliminated from the VAS and should now be addressed in the Research Strategy as part of experimental design.

All experiments involve mice of the C57BL/6 background.

For each cell-specific-RiboTag mouse cohort, there will be n=15 mice per group, at 3 ages (Young, Midlife, Old) and 2 sexes (female, male) with two disease states (EAE/Health), this will require n=180 total mice (15x3x2x2=180). This cohort (n=180) of mice will have mice n=15 mice removed at each age (Young, Midlife, Old) from each sex (female, male) for EAE or Healthy control to assess brain atrophy and neuropathology outcomes.

The *in vivo* translatome of each cell type will be determined by using RiboTag mice crossed with CNS cell-specific promotor driven, tamoxifen inducible lines. Tamoxifen or vehicle treatment at age 1.5 months (by i.p. injection on 5 consecutive days). Gene expression will be determined during young adulthood (3-4 months) and midlife (12-14 months) and old (20-22 months) in a cell-specific, region-specific and sex-specific manner).

For cell-specific target gene deletion (for example, *Kdm6a* CKO and WT mice) experiments: there will be sample size of n=15 in each group, 2 ages (3mo, 18 mo), 2 genotypes (CKO, WT), 2 sexes (female, male) and 2 clinical states (health, EAE), this experiment will require a total of 240 mice. Brain MRI will be done 40 days after EAE induction (including in age matched healthy controls), and tissues will be collected from CNS regions as described for neuropathology.

Pharmacologic treatment (metformin for example) versus placebo treatment will determine the effect of treatment on disease (clinical, regional brain atrophy, and neuropathology). Treatment of cell-specific RiboTag mice will reveal how the treatment impacts cell-specific gene expression in vivo. For example, metformin will also be used in *Kdm6a*-cell specific CKO crossed with RiboTag mice to reveal how the *Kdm6a* CKO affects the cell-specific translatome. Two weeks after the last tamoxifen injection (age 2 months), daily treatment with 100 mg metformin or control i.p. injections will begin.

Note that over the course of this 8 year grant, optimal gene deletion targets and pharmacologic treatments will evolve, so all cannot be predicted at present, however the same approach as described above for *Kdm6a* and metformin will be used.

No dogs or cats.

2. Justifications

Provide justification that the species are appropriate for the proposed research. Explain why the research goals cannot be accomplished using an alternative model (e.g., computational, human, invertebrate, in vitro).

The murine models of aging are widely used for assessing neurodegeneration. The C57BL/6 mouse is particularly useful in light of the genetic modifications and reagents that exist. To date, there is no other phylogenetically lower species which models these aspects. Additionally, in vitro and computer models do not mimic the complexity of mammalian physiology, so information from these models would not as fully recapitulate aging. Our ultimate goal is to lay a foundation for the future that will use the model to develop and test novel therapeutic strategies in patients.

The murine models of MS are widely used for assessing neuroinflammation. Our ultimate goal is to use the model to develop novel therapeutic strategies for MS patients. If we do not test these agents in the murine MS model first, clinical trials in MS proposals will not be funded due to lack of "preclinical data". New treatments must be effective at minimum in mice with EAE first before translating to humans. Finally, conditional knocks of *Kdm6a* in mice will allow us to understand the role of sex chromosomes in neuroinflammation.

3. Minimization of Pain and Distress

Describe the interventions to minimize discomfort, distress, pain, and injury. These include analgesia, anesthesia, sedation, palliative care, and humane endpoints.

Mice are cared for by a licensed veterinarian (Division of Laboratory Animal Medicine, UCLA). With regard to pain and distress, EAE mice will be monitored in accord with UCLA DLAM guidelines.

With regard to pain and distress, all mice without EAE are in Category A, while all mice induced to have EAE are in Category C regardless of how mild their disease is. EAE is characterized by temporary paralysis, with no evidence of pain based on the human demyelinating CNS disease multiple sclerosis. Mice with EAE are checked twice daily and this data is recorded for each mouse. Mice with severe EAE, grade 3 or 4 are housed separately from mice with less severe EAE to avoid competition for food and hydration as well as to avoid harm of the weaker mice by the stronger mice. If during a particular time period, the mice become too weak to reach the water supply, apples are routinely added to cages and subcutaneous injections of PBS are given when needed.

The procedures which require **anesthesia** include the following.

Active EAE induction: Mice are first anesthesized with 2-3% isoflurane gas mixed with oxygen using a vaporizer (Summit, Model:PAM). The injections to induce EAE are then given.

Upon completion of experiments or if EAE should become severe enough to produce a moribund state, mice are **euthanized** by isoflurane inhalation using a nose cone.

Mice will undergo brain MRIs *in vivo* in a 7 T Bruker spectrometer under isoflurane anesthesia. Prior to imaging the mice will be brought to a plane of anesthesia where they are not responsive to a toe pinch and then placed in a head restraint within the imaging cradle. If the animal appears to be dehydrated, a 0.5 mL bolus of sterile saline solution will be administered subcutaneously. The imaging cradle will be maintained at 37° C and respiration will be monitored continuously during the scan. If the animal appears to be in distress (very slow or very fast respiration), the imaging session will be aborted, the mouse removed from the head restrain and imaging cradle and also anesthesia. Mice will recover from anesthesia on a warming pad and will be monitored continuously until conscious and then returned to their cage.

Upon completion of experiments, mice are **euthanized** by isoflurane inhalation using a nose cone.

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July 8, 2022

Chief Grants Management Officer
National Institute of Neurological Disorders and Stroke (NINDS)
ChiefGrantsManagementOfficer@ninds.nih.gov

Dear Chief Grants Management Officer,

On behalf of The Regents of the University of California, I am pleased to submit and support the enclosed proposal:

Title:	Neurodegeneration Underlying Distinct Disabilities in Multiple Sclerosis Using a Cell-Specific, Region-Specific, and Sex-Specific Approach
Principal Investigator:	Rhonda Voskuhl, MD
Project Period:	04/01/2023 through 03/31/2031
Amount Requested:	$7,307,976

The University is aware of and accept the condition that other NINDS research awards must be relinquished as a condition of receiving the NINDS Research Program Award (RPA). If awarded, Dr. Voskuhl will devote 6.60 person months to the RPA throughout the duration of the award period.

If you have any questions or require additional information, please contact us at your convenience.

Sincerely,

Digitally signed by
Jessica Kim
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July 6, 2022

Dear Colleagues,
It is pleasure to write this letter of support for Dr. **Rhonda Voskuhl** for her R35 application. As Chair of Neurology, I have worked closely with Rhonda in her Departmental and scientific leadership and building of an impressive multiple sclerosis program. As physician-scientist, I have collaborated with Rhonda in her scientific studies, and know her bench and clinical research and its impact. Dr. Voskuhl has pioneered several important directions in MS research, translated these into clinical trials and has mentored young faculty toward successful careers.

Rhonda is a leader in the field of sex differences in disease, showing sex chromosome and sex hormone effects in autoimmunity and neurodegeneration. This work started with the innovative sex chromosome models in mice, and findings in both MS and lupus in an effect on disease pathogenesis. She then identified a specific gene that mediated the susceptibility to autoimmune disease on the X chromosome. This work was published in high impact journals and rigorously pursued the research issues of sex chromosome effects down to the molecular level. Along the way, and in a particularly surprising set of findings, Rhonda's lab showed that the XY chromosome complement leads to enhanced neurodegenerative disease in the same model systems in which XX leads to an increase in autoimmune disease. This work has clear implications for MS clinically.

Rhonda Voskuhl used similarly innovative approaches to identify cell-specific and region-specific differences in transcriptomics in the CNS response to injury. I remember very well when these studies started to really take off. Rhonda had the ideas that MS pathogenesis would involve key components from astrocyte signaling and responses, and initiated a collaboration with Dr. Michael Sofroniew to pursue this idea. At the time Michael had developed astrocyte-specific transcriptional profiling that was not widely available in the field, and was studying spinal cord injury. Rhonda's initiative took these tools in studies of MS, and identified several important astrocyte metabolic and signaling pathways that associate with disease severity or progression. In further studies, some of these molecular pathways in astrocytes and also in oligodendrocytes, segregate by brain region in the MS model. These findings suggest novel treatments that might target distinct effects of damage and secondary disabilities aligned by brain region in multiple sclerosis.

A particularly strong example of Rhonda's role in the MS field and impact as a physician-scientist is in her work in estrogen effects in this disease. She characterized the effects of selective estrogen receptor pathway signaling in MS, and identified estrogen receptors as promising targets—specifically in astrocytes, then in oligodendrocytes. As with her other work, these studies were published in high impact journals, and were done in parallel projects in her lab to those noted above. Rhonda took this emerging estrogen story and moved it toward the clinic with estriol—a molecule that was deliberately chosen because it is the estrogen unique to pregnancy, a time of protection in MS, as well as being inexpensive and readily available across global economic strata. Rhonda developed a multi-site clinical trial to test estriol. She applied quantitative brain imaging and found a difference in effect by disability measure and brain region. In recent work, she has developed patterns of regional brain atrophy together with serum neurofilament light chain as biomarkers for further clinical trials.

In the course of this work, Rhonda has patented many of the approaches, such as one set of patents that is licensed pertaining to estriol treatment of cognitive decline in menopausal women and another group which entails use of estrogen receptor beta selective ligand treatment of multiple sclerosis. This has had the important effect of stimulating pharmaceutical company interest in moving these therapies forward on a larger scale.

In the midst of this scientific work, Dr. Voskuhl has run the MS clinical program at UCLA, and mentored many MS fellows. She has recruited two very talented junior faculty to UCLA, Kevin Patel and Elaine Su, to grow specific aspects of the MS program in imaging and clinical trial approaches. Rhonda is a strong leader in the Department, and a voice for bench-to-bedside clinical translation, in setting up infrastructure and faculty mentoring that will facilitate basic and clinical research.

In short, Rhonda Voskuhl is an excellent candidate for the R35 program for her superb and wide-ranging research, leadership of her field, identification of new biological principles in autoimmunity and neurodegenerative diseases and growth of the field through mentorship and a vision for translational science.

S. Thomas Carmichael, M.D., Ph.D.
Professor and Chair
Frances Stark Chair
Department of Neurology
David Geffen School of Medicine at UCLA

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STATISTICS CORE
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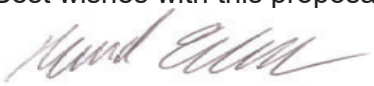
June 26th, 2022

Dear Rhonda,

I am writing to express my support for your NINDS NIH R35 application entitled. "Neurodegeneration underlying distinct disabilities in multiple sclerosis using a cell-specific, region-specific, and sex-specific approach." As the Director of Department of Medicine Statistics Core (DOMStat), I am committed to providing the resources needed for statistical analyses of your gene expression data. Dr. Jin Zhou, Associate Professor Department of Medicine Statistics Core, has substantial expertise in genetics and genomics. As you know, UCLA is a leader in genomics research, and Dr. Zhou's experience in metagenomics data analysis makes her a good fit for your proposal. She is available to collaborate with you on this interesting project integrating transcriptomics analyses across brain cell types, regions, sex, and age. The level of her involvement would be commensurate with Dr. Zhou being a Co-Investigator, and we could add additional staff statisticians as needed from DOMStat.

Best wishes with this proposal,



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July 4, 2022

Dear Rhonda,

This is a Letter of Support for your R35 grant to NINDS, NIH entitled, "Neurodegeneration underlying distinct disabilities in multiple sclerosis using a cell-specific, region-specific, and sex-specific approach". Given that this grant application includes both an MS model for preclinical research as well as MS human data for clinical research, its human MS aspect aligns well with the going collaboration between my team at Charité, Universitätsmedizin Berlin and your team at the University of California, Los Angeles (UCLA).

As you know, I am fully supportive of our ongoing collaboration which has entailed transfer of data from an existing dataset of MS patients at Charité, Universitätsmedizin Berlin to UCLA. Dr. Allan MacKenzie-Graham of your group is determining region-specific and sex-specific gray matter atrophy in female and male MS patients using data from my existing longitudinal dataset of very high quality clinical and imaging data collected from 280 subjects (112 female MS patients and 99 male MS patients matched for age and disease duration, plus 43 female healthy controls and 26 male healthy controls also matched for age) followed for up to five years by my MS group at Charité – Universitätsmedizin Berlin.

Our collaboration has been successful, with initial cross-sectional findings in a smaller dataset published ("Sex differences in brain atrophy in multiple sclerosis", *Biology of Sex Differences*, 11:49, 2020). I am enthusiastic as we expand our collaboration into regional differences in sex differences in our ongoing larger, longitudinal study, with ultimate integration of findings in human MS with sex-specific, region-specific, and cell-specific differences in gene expression and neuropathology in the most widely used MS animal model, experimental autoimmune encephalomyelitis (EAE).

Best wishes for success in your R35 grant application, which is central to a precision medicine approach to better understand neurodegeneration in MS.

Sincerely,

Friedemann Paul, MD

Data Sharing Plan:

The proposed research will include data from genome wide transcriptome analysis of specific CNS cell types in chronic EAE, health, and aging from both sexes (female and male) of mice. Data generated from use of the chronic cuprizone model will also be shared. Select groups will also have methylome data available.

For data sharing purpose, all high throughput data will be deposited in GEO (Gene Expression Omnibus, NCBI) database and will be publicly available.

Mice created during the course of this grant through crosses and breeding will be made available to other researchers upon request.

At this point, it is difficult to predict precisely what types of discoveries made during analyses of such sequences may have intellectual property implications. If the results produced within this proposal have potential as a biomarker or targeted drug therapy, we will discuss the intellectual property issues with a representative from the UCLA Office of Intellectual Property. Regarding patentable inventions, the title will belong to UCLA, with nonexclusive, royalty-free license to the Government per 37 CFR 401.14, “Patent Rights (Small Business Firms and Nonprofit Organizations)”.

Voskuhl Authentication of Key Reagents:

This grant does not use cell lines or specialty chemicals.

Antibodies and other biologics are commercially available and commercially validated.

Experiments involving Cre-loxP recombination using Ribotag mice to HA label RNAs from each cell type are generated with mice currently commercially available (Jackson labs). As outlined in our research plan, all mice have been and will continue to be validated by genotyping and immunohistochemistry. Also, cell-specific RNA enrichment is validated using double label immunofluorescence immunostaining and qPCR for enrichment of targeted cell type and de-enrichment of other cell types (as described in our previous publications).